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Calcium Signaling and Cardiac Arrhythmias

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Abstract

There has been significant progress in our understanding of the molecular mechanisms by which calcium (Ca^{2+}) ions mediate various types of cardiac arrhythmias. A growing list of inherited gene defects can cause potentially lethal cardiac arrhythmia syndromes, including catecholaminergic polymorphic ventricular tachycardia, congenital long QT syndrome, and hypertrophic cardiomyopathy. In addition, acquired deficits of multiple Ca^{2+}-handling proteins can contribute to the pathogenesis of arrhythmias in patients with various types of heart disease. In this review article, we will first review the key role of Ca^{2+} in normal cardiac function - in particular, excitation-contraction coupling and normal electrical rhythms. The functional involvement of Ca^{2+} in distinct arrhythmia mechanisms will be discussed, followed by various inherited arrhythmia syndromes caused by mutations in Ca^{2+}-handling proteins. Finally, we will discuss how changes in the expression of regulation of Ca^{2+} channels and transporters can cause acquired arrhythmias, and how these mechanisms might be targeted for therapeutic purposes.

Subject codes

Arrhythmias; Calcium Cycling/Excitation-Contraction Coupling; Genetics; Cardiomyopathy

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DISCLOSURES

X.H.T.W. is a founding partner of Elex Biotech, a start-up company that developed drug molecules that target ryanodine receptors for the treatment of cardiac arrhythmia disorders.

Keywords

Atrial fibrillation; arrhythmias; calcium channels; RyR2; ventricular tachycardia

The bivalent cation calcium (Ca^{2+}) represents one of the most ubiquitous signal transduction molecules known.[1] It mediates a diverse array of biological functions including the muscle contraction, cellular exocytosis, neuronal activity, and triggering of programmed cellular death. Since the first observation by Ringer in 1883 that Ca^{2+} was required for cardiac contraction, the role of Ca^{2+} as a signaling ion in the heart has become increasingly appreciated.[2] In addition, it has become clear that abnormalities of Ca^{2+} homeostasis can play a key role in the pathogenesis of common cardiovascular disorders, including cardiac arrhythmias. Human genetic studies of patients with inherited arrhythmia syndromes have uncovered inherited mutations in various Ca^{2+} channels and Ca^{2+} transporters, directly implicating dysfunction of these proteins in the disease mechanisms. Moreover, acquired modifications of various Ca^{2+}-handling proteins have been associated with cardiac arrhythmias, including atrial fibrillation (AF) and ventricular arrhythmias in failing hearts. In this review, we provide a comprehensive overview of the potential contributions of Ca^{2+} in arrhythmia mechanisms, and highlight various gaps in knowledge and controversies in the field.

OVERVIEW OF EXCITATION-CONTRACTION COUPLING IN THE HEART

Regular contraction of the heart requires the conversion of electrical activation (excitation) into mechanical force (contraction). This process, known as excitation-contraction (EC) coupling, requires coordinated movement of Ca^{2+} ions at the cardiomyocyte level (Figure 1A). Each action potential (AP), triggered by influx of sodium (Na^+) through the voltage-gated sodium channel (Nav1.5), thereby generating the I_{Na} current, induces Ca^{2+} influx through voltage-activated L-type Ca^{2+} channels (LTCCs, Cav1.2), creating the $I_{Ca,L}$ current. This Ca^{2+} triggers a much larger Ca^{2+} release from the sarcoplasmic reticulum (SR), the principal intracellular Ca^{2+} storage organelle.[3] SR Ca^{2+} release is mediated by specialized Ca^{2+}-release channels known as ryanodine receptor type-2 (RyR2).[4] This process of Ca^{2+}-sensitive RyR2-mediated SR release is known as Ca^{2+}-induced Ca^{2+}-release (CICR). The cytosolic Ca^{2+} binds to and activates cardiac troponin C (TnC), the Ca^{2+}-sensing protein of the contractile apparatus, and initiates myofilament contraction. During diastole, cardiac muscle relaxation occurs when Ca^{2+} is removed from the cytosol either by sequestration into the SR by the SR Ca^{2+}-ATPase type-2a (SERCA2a) or out into the extracellular space by the Na^+/Ca^{2+}-exchanger type-1 (NCX1). In addition, there is a minor contribution by the plasmalemmal Ca^{2+}-ATPase (PMCA). NCX1 is electrogenic, as it imports three Na^+ ions into the cell for each extruded Ca^{2+} ion, thereby creating a depolarizing transient inward current (I_{NCX}). The rapid release of Ca^{2+} from the SR into the cytosol, followed by rapid reuptake into the SR or extrusion from the cell, creates a Ca^{2+} wave that runs through the cardiomyocyte and is known as the Ca^{2+} transient. The amount of Ca^{2+} released from the SR via RyR2 largely determines the Ca^{2+}-transient amplitude, which correlates with the strength of systolic contraction.

EC coupling occurs within specialized subcellular structures called junctional membrane complexes (JMCs), where LTCCs on transverse T-tubules – plasmalemmal invaginations that reach deep into myocytes – are positioned in close proximity of the RyR2 channels on the SR membranes (Figure 1B).[5] The movement of Ca^{2+} within these dyadic cleft domains is, in part, regulated by junctophilin-2 (JPH2), a protein that provides a structural bridge between the plasmalemma and SR ensuring appropriate proximity between the LTCC and RyR2 channels[6, 7]. JPH2 is also necessary for bridging integrator 1 (BIN1) recruitment to develop the T-tubule forming the dyad. There are important differences in the organization of the JMC between atrial and ventricular cardiomyocytes.[8] In ventricular myocytes, almost all Ca^{2+} release events (i.e., sparks and transients/waves) are activated directly by LTCC on T-tubules which leads to synchronized SR Ca^{2+} release and a rapid upstroke of the Ca^{2+} transient. In atrial cardiomyocytes, in which TTs are relatively underdeveloped, the Ca^{2+} transient begins with LTCC-triggered local SR Ca^{2+}-release events at the cell periphery that propagate slowly as Ca^{2+} waves towards the cell center.[9, 10] In addition, atrial cardiomyocytes possess larger and more heterogeneous axial tubules and much more Ca^{2+}-buffering mitochondria than ventricular cardiomyocytes.[11, 12] Finally, another class of Ca^{2+} release channels known as inositol 1,4,5-trisphosphate type 2 receptors (IP_3R2) may also contribute to CICR.[13]

REGULATION OF INTRACELLULAR CALCIUM-HANDLING

The activity of Ca^{2+} channels and exchangers involved in EC coupling is regulated by several mechanisms and signaling pathways in response to changing demands for cardiac output. For example, the ‘fight-or-flight’ response activates the sympathetic portion of the autonomous nervous system with downstream effects on Ca^{2+} signaling (recently reviewed).[14] Activation of the β-adrenoceptor (βAR) causes a rise in the intracellular concentration of the second messenger cyclic adenosine monophosphate (cAMP). Downstream effectors of cAMP include cAMP-dependent protein kinase A (PKA), which in turn can phosphorylate Ca^{2+} transporters including LTCC, RyR2, and SERCA2a regulatory proteins like phospholamban (PLN) and sarcolipin (SLN). In addition, the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) can modulate Ca^{2+} homeostasis in response to changes in heart rate, cellular oxidation levels, and persistent βAR stimulation.[4, 15]

Each of the Ca^{2+} channels and transporters consist of pore-forming proteins and various accessory subunits that modulate the amount of Ca^{2+} that is moved through the pore. These channels and exchangers have been extensively reviewed elsewhere.[16, 17] Perhaps one of the most well studied multiprotein complexes is the RyR2 macromolecular complex. A diverse array of RyR2-interacting proteins directly regulate RyR2 channel activity by binding to the pore subunit (e.g., FK506-binding protein-12.6 (FKBP12.6), calmodulin (CaM), calsequestrin-2 (CASQ2), junctin (JCTN), triadin (TRDN), βII-spectrin (Figure 2).[18, 19] CASQ2 binds to RyR2 via both JCTN and TRDN. RyR2 is strongly regulated by luminal (within the SR) Ca^{2+} levels, either by direct Ca^{2+} binding to RyR2, or by luminal Ca^{2+} interacting with CASQ2, JCTN, and TRDN.[20] Other proteins in the RyR2 macromolecular complex regulate the level of RyR2 posttranslational modification. Examples include the protein kinases PKA, CaMKII, and newly discovered striated preferentially expressed protein kinase (SPEG), and protein phosphatases type-1 and type-2A (PP1, PP2A), that

regulate the actual level of RyR2 phosphorylation. [21–24] The RyR2 channel is also regulated by S-nitrosylation and oxidation.[25]

The LTCC, responsible for voltage-dependent Ca^{2+} entry into the cells, consists of a macromolecular protein complex comprised of pore-forming Cav1.2 (β subunit) and various auxillary subunits (β2, β, δ, and γ) that modulate channel function (Figure 3). Similar to RyR2, the LTCC is regulated by protein kinases such as CaMKII and PKA as well as protein phosphatases PP1, PP2A and calcineurin (also known as protein phosphatase 2B, PP2B), which can modulate channel gating. In addition, regulatory subunits, like CaM, are embedded within the channel complex.[26] LTCC are localized to rafts of other sarcolemmal ion channels and membrane-limited proteins.[27, 28] A critical mediator of this membrane clustering is caveolin 3 (CAV3) which binds and interacts with the N-terminal part of JPH2.[29]

Finally, SERCA2a is a macromolecular complex required for Ca^{2+}-reuptake into the SR (Figure 4). It is allosterically regulated by phosphorylation-mediated conformational shifts of its regulatory subunit PLN, that can be phosphorylated by PKA and CaMKII.[30, 31] These post-translational modifications can relieve the PLN-mediated inhibition of SERCA2a, allowing for rapid Ca^{2+} reuptake. Histidine-rich Ca^{2+}-binding protein (HRC) has been shown to bind the SR luminal side of SERCA2a and to interact with TRDN, potentially coordinating Ca^{2+}-reuptake with SR Ca^{2+}-release along with S100A1.[32, 33] Moreover, calreticulin (CALR) may play a role in the inactivation and degradation of SERCA2a under oxidative stress.[34]

FUNDAMENTAL ARRHYTHMIA MECHANISMS

The mechanisms responsible for cardiac arrhythmias are generally divided into two major categories – enhanced or abnormal impulse generation (i.e., focal activity), and conduction disturbances (i.e., reentry).[35, 36] Focal activity includes enhanced automaticity and triggered activity. Automaticity causes spontaneous generation of APs that do not require induction by previous beats. Healthy myocardium is not normally automatic, but disease conditions (e.g. heart failure, HF) can lead to resting membrane potential depolarization to more positive values causing abnormal automaticity. The most common causes of focal arrhythmias are early afterdepolarizations (EADs) that precede full repolarization (typically corresponding to phase-2 and phase-3 repolarization of the human AP) and delayed afterdepolarizations (DADs) that occur after full repolarization.

EADs cause focal firing by depolarizing surrounding tissue to excitation threshold EADs and are most characteristic of Purkinje-fiber tissue and ventricular tachyarrhythmias associated with HF and long-QT syndrome (Figure 5A). EADs are usually, but not exclusively, associated with excessive AP prolongation (e.g. by increased inward $I_{Ca,L}$ [37] and late Na^+-current $I_{Na,L}$ or I_{NCX},[38, 39] or by reduced K^+-currents (I_K), allowing $I_{Ca,L}$ to recover from inactivation and depolarize the cardiomyocyte by allowing Ca^{2+} to enter.[40] CaMKII-dependent $I_{Ca,L}$ phosphorylation slows inactivation and accelerates recovery from inactivation, further enhancing the likelihood of EADs.[37] At membrane potentials negative to the threshold of $I_{Ca,L}$ activation, spontaneous SR Ca^{2+} release-activated NCX favors the

non-equilibrium reactivation of I_{Na}, driving phase-3 EADs induction.[41, 42] Finally, EADs have also been associated with APD shortening, occurring late in phase-3 of the AP.[43] If the intracellular Ca^{2+} concentration is still high (e.g., due to a large Ca^{2+} transient) when the membrane potential is negative to the equilibrium potential for NCX, I_{NCX} can be activated leading to membrane depolarization. This type of EADs typically occurs after termination of ventricular tachycardia (VT), ventricular fibrillation (VF), and AF. Overall, in regions where EADs reach the threshold to propagate, they generate triggers that initiate reentry.

DADs typically occur during diastole and conditions of elevated cellular Ca^{2+}-loading (Figure 5B). They are caused by spontaneous rises in cytoplasmic Ca^{2+}-concentration, which activate NCX, generating forward mode I_{NCX}, although other Ca^{2+}-sensitive currents (nonselective cationic currents and chloride currents) might also contribute to DAD formation.[44] The amplitude of the DAD depends on the size of the resting K^+ conductance, mainly determined by the inward-rectifier K^+-current I_{K1}, relative to I_{NCX} amplitude. When I_{K1} is low, the same I_{NCX} will produce a larger DAD and *vice versa*.[45] When DADs reach excitation-threshold, I_{Na} is activated and spontaneous APs can arise. DAD-mediated triggered activity contributes the arrhythmogenesis associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), HF and AF.

Reentry can occur around a fixed anatomical obstacle or in a substrate in which functional properties permit initiation and maintenance of reentrant circuits.[46] The likelihood of reentry formation is determined by the tissue properties of conduction and refractoriness, with abnormal conduction (slowing and/or local block) and refractoriness (abbreviated or prolonged) making reentry more likely (see Figure 5C–D). Refractory period depends on AP duration (APD), whereas conduction velocity largely depends on I_{Na}, expression and localization of gap-junction proteins, and composition of extracellular matrix (e.g. fibrosis). When the refractory period decreases (like in AF), the circuits are smaller and more numerous, simultaneous termination of all circuits is unlikely and the arrhythmia is sustained. When the refractory period is prolonged (like in HF), the heterogeneity (dispersion) of refractoriness is increased and the occurrence of reentry promoting conduction block is more likely. The reentry-promoting substrate can be caused by disease-related cardiac remodeling or predisposing genetic factors, but can also be produced by altered restitution dynamics and subcellular Ca^{2+}-alternans (SR Ca^{2+} load and release alternans).[47] Altered Ca^{2+} signaling can contribute to the formation of a reentry substrate by two mechanisms: promoting dispersion of excitability, and promoting dispersion of refractoriness.[35] For example, DADs that do not reach the threshold to trigger an AP can cause resting membrane potential depolarization, increasing Na^+-channel inactivation and promoting dispersion of excitability. The latter might lead to regional conduction block of impulses arising from regions with supra-threshold DADs, thereby promoting reentry initiation. EADs that remain below the threshold to propagate may increase dispersion of refractoriness, also creating a reentry substrate. The rapid rates during DAD-induced triggered activity can promote Ca^{2+} transient alternans, which can cause spatially discordant APD alternans, thereby enhancing the dispersion of refractoriness and the likelihood of reentry.[48] Thus depending on the cellular and tissue context, EADs, DADs and Ca^{2+} alternans can provide the trigger and may contribute to the formation of the reentry-promoting substrate. A deep understanding of the detailed molecular mechanisms by which

abnormal Ca^{2+}-signaling increases the susceptibility to cardiac arrhythmias is key for the development of novel therapeutic options for prevention and treatment of cardiac arrhythmias.

ARRHYTHMIAS CAUSED BY HERITABLE DEFECTS IN CALCIUM-HANDLING GENES

The discovery of the first inherited mutations in genes encoding Ca^{2+}-regulatory proteins has provided the best evidence to date that defects in intracellular Ca^{2+}-handling can directly cause different cardiac arrhythmias (Figure 6). In the following section, we will review several inherited arrhythmia syndromes that are often caused by mutations in genes encoding Ca^{2+} channels, transporters, or related proteins.[49]

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

The inherited arrhythmia disorder CPVT is one of the most-deadly arrhythmias known, and it classically manifests with βAR-induced syncope or sudden cardiac death (SCD).[50, 51] CPVT was first described in 1978 as a distinct syndrome associated with syncope and arrhythmia in the setting of a structurally normal heart. This condition can present with premature ventricular contractions (PVCs) at rest, or with exercise, and this ventricular ectopy can degenerate into bidirectional VT or VF. The majority of CPVT cases present in childhood and have normal cardiac repolarization on ECG measurement of the QT interval.[52] The estimated prevalence of CPVT is around 1:5,000 to 1:10,000 depending on the population studied.[53]

RYR2-encoded ryanodine receptor type-2 (CPVT-1)—In the first major case series of children described to have CPVT, the authors noted the presence of bidirectional VT as an arrhythmia previously often associated with digitalis toxicity.[52] This led to the hypothesis that the etiology of CPVT may be due to DADs induced by increased SR Ca^{2+} load exacerbated by catecholamines.[54] A genetic locus associated with CPVT was first mapped to 1q42-43 of the genome in a large study of two unrelated families with a heritable, autosomal-dominant syndrome manifesting as stress-induced polymorphic VT, syncope, and SCD with structurally normal hearts.[55] Two years later, inherited mutations in the *RYR2* gene were identified as the most common genetic subtype of CPVT (CPVT-1; Table 1).[56, 57]

Subsequent studies rapidly expanded the spectrum of *RYR2* mutations in CPVT which account for approximately 50 to 60% of all cases.[56] Pathogenic mutations most often alter a single amino acid (missense mutations) and are inherited in autosomal-dominant pattern. CPVT-associated mutations in *RYR2* almost always result in increased SR Ca^{2+} leak which is amplified in the setting of increased sympathetic drive.[58] This increased propensity to SR Ca^{2+} leak can be detected as an increase in the frequency of elementary Ca^{2+}-release events (i.e., Ca^{2+} sparks).[59] It is believed that diastolic SR Ca^{2+} leak can lead to increased intracellular Ca^{2+} which activates NCX during diastole, leading to DADs and triggering of ventricular arrhythmias.[60] Several aspects of the pathophysiology of CPVT caused by RyR2 mutations remain controversial, including the potential role of reduced binding of FKBP12.6 to RyR2, channel gating deficits in the absence of βAR stimulation, and the potential

involvement of SR Ca^{2+} overload as an additional mechanism. For example, the role of FKBP12.6 in regulating RyR2 Ca^{2+}-release and the role of PKA-mediated phosphorylation on RyR2 in cardiac arrhythmia and HF are subjects of on-going debate.[61]

Early studies demonstrated that FKBP12.6 was expressed in the heart, associated with RyR2, and modulated CICR.[62] Further, studies found that FKBP12.6 directly bound RyR2 and stabilized the closed conformational state of the protein such that removal caused SR Ca^{2+} leak[63, 64]. This stabilizing property of FKBP12.6 was not universally observed.[65]. As this line of exploration was developing, a separate body of evidence was emerging that RyR2 phosphorylation at serine 2808 (S2808) by PKA could increase channel opening probability as part of the "fight or flight" mechanism.[66, 67] These studies converged with the observation that PKA-mediated increased channel sensitivity to Ca^{2+} was based on partial dissociation of FKBP12.6 binding following S2808 phosphorylation, and identified lethal exercise-induced arrhythmias in FKBP12.6 knockout mice (Fkbp12.6−/−).[58] This observation was expanded to other forms of cardiac disease, including HF, whereby elevated βAR signaling through PKA resulted in hyperphosphorylated S2808 and dissociation of FKBP12.6.[68, 69] These findings have not been universally observed by other investigators have catalyzed a number of follow-up studies which have introduced debate in the field.[70, 71] Some have argued that reduced Ca^{2+} reuptake into the SR led is the predominant mechanism underlying HF[72] or that PLN activity and increased SR Ca^{2+} load is involved.[73] There is also evidence that CaMKII phosphorylation of RyR2 may contribute to the development of HF and arrhythmogenesis through increased Ca^{2+} leak.[74] For in-depth review of this topic, please refer to prior articles.[75–77] Overall, these studies highlight the complexity of Ca^{2+} release regulation in the cardiac myocyte.

Studies of several knock-in mouse models of human *RYR2* mutations have provided additional insights into the pathogenesis of CVPT.[59, 78–80] Based on some of these studies, it has been proposed that Purkinje cells in a mouse model of CPVT exhibited a higher frequency and amplitude of spontaneous SR Ca^{2+}-release events, suggesting that focal arrhythmias might originate from the specialized conduction system.[81] More sophisticated genetic studies are needed to confirm whether Purkinje cells are truly the source of triggered arrhythmias in CPVT mutant mice as well as in patients with this condition. Finally, recent studies in human induced pluripotent stem cells (iPSC) have confirmed prior studies on recombinantly expressed channels and studies in mouse models, while providing additional mechanistic insights. For example, it has been shown that iPSC-derived cardiomyocytes (iPSC-CM) from CPVT patients exhibit an increased susceptibility to DADs due to abnormal SR Ca^{2+}-release events[82]. Overall, these studies demonstrate that exacerbation of DADs following sympathetic stimulation is the key mechanism and that β-blockers, dantrolene, CaMKII inhibitors like KN-93s, and RyR2-inhibiting compounds such a S107 all represent potential therapeutic options for CPVT.[82–84] Subsequent clinical studies in CPVT patients confirmed the anti-arrhythmic potential of dantrolene.[85] Thus, iPSC-CM from CPVT patients may represent a valuable system for preclinical drug screening.

CASQ2-encoded calsequestrin type-2 (CPVT-2)—A second rare genetic subtype of CPVT (CPVT-2) is caused by autosomal-recessive variants in *CASQ2*-encoded CASQ2, the most abundant Ca^{2+}-buffering protein in the SR. These mutations are relatively rare among

CPVT cases, accounting for only about 3–5% of all patients with CPVT.[86] This genetic subtype, initially identified in 7 families of a Bedouin tribe in northern Israel, is characterized by resting bradycardia and VT by treadmill or βAR activation with isoprenaline infusion.[87] Recently, a family with a unique autosomal-dominant form of CPVT was found to be caused by a *CASQ2* mutation.[88]. CASQ2 is the cardiac-specific isoform of a family of proteins which directly and indirectly regulate SR Ca^{2+} storage and release.[89] CASQ2 has a high-binding capacity (40–50 mol of Ca^{2+}/mol) but a moderate affinity (Kd of 1 mM) for free Ca^{2+} and serves as molecular sink for Ca^{2+} that has been sequestered into SR following cardiac contraction.[90] With an increased prevalence of acidic amino acid residues, it is believed that the negatively charged CASQ2 directly binds free Ca^{2+}.[91]

All *CASQ2* mutations identified so far are missense, deletion, or nonsense mutations which lead to a severe reduction, or complete loss of, the CASQ2 protein.[92] RyR2 channels that lack CASQ2 open spontaneously without being triggered by $I_{Ca,L}$-mediated Ca^{2+} influx [93] Studies in isolated rat cardiomyocytes transfected with mutant CASQ2 protein revealed a reduced SR store Ca^{2+} capacity with spontaneous Ca^{2+} transient generation and evidence of DADs.[94] This effect was abrogated by addition of citrate, a low-affinity Ca^{2+} buffer, suggesting that mutant CASQ2 destabilizes SR-store Ca^{2+} capacity which alters the Ca^{2+}-sensitivity of RyR2 resulting in pro-arrhythmic DADs.[94]. Other studies utilizing knock-in mice carrying missense or radical loss-of-function human mutations demonstrated reduced CASQ2 expression with elevated resting cytosolic Ca^{2+} levels and reduced SR-store Ca^{2+} which was further exacerbated by βAR stress.[95] CASQ2 is part of the RyR2 macromolecular complex which also involves the SR-proteins TRDN and JCTN.[96, 97]. The levels of these proteins are often dramatically altered when *CASQ2* is genetically ablated or mutated.[96, 98] Moreover, increased levels of CALR and RyR2, which increases SR Ca^{2+} leak have been reported in mice with mutant *CASQ2*.[95] Therefore, it cannot be excluded that some of the RyR2 functional changes in *CASQ2* mutant mice are, at least in part, mediated by changes in TRDN, JCTN, or CALR levels. Finally, the mechanisms of increased arrhythmogenesis have been confirmed in human iPSC-CM. For instance, the βAR agonist isoprenaline caused DADs, oscillatory arrhythmic prepotentials, and after-contractions in cardiomyocytes derived from CPVT patients with *CASQ2* variants but not from individuals with normal *CASQ2*.[99, 100]

TECRL-encoded trans-2,3-enoyl-CoA reductase-like protein (CPVT-3)—A third genetic subtype of CPVT is the gene encoding trans-2,3-enoyl-CoA reductase-like protein (TECRL). Initially identified by linkage analysis in a consanguineous Sudanese family with multiple SCDs among children while playing, subsequent whole exome sequencing (WES) identified mutations in a handful of families and probands in *TECRL*.[101,102] Each patient demonstrated VT and VF, particularly with exertion, and had SCD. Interestingly, while the subjects had normal QT intervals at baseline, adrenergic stimulation caused QT interval prolongation. As such, mutations in the *TECRL* gene appear to cause an overlap syndrome with features clearly associated with CPVT but also congenital long QT syndrome (LQTS, see below).

Creation of a mouse model of *TECRL* mutations is necessary to examine arrhythmia mechanisms in the experimental setting. Studies of iPSC-CM generated from the Sudanese proband demonstrated reduced systolic Ca^{2+}-transient amplitudes and reduced caffeine-stimulated Ca^{2+} transient amplitudes (an index of SR Ca^{2+} content) along with elevated resting cytosolic Ca^{2+} levels, consistent with presence of SR Ca^{2+} leak as seen in CPVT-1 and CPVT-2.[82, 99] In addition, mutant iPSC-CMs demonstrated slower Ca^{2+}-transient upstroke velocity and impaired SR Ca^{2+}-reuptake when compared to both heterozygous and wild-type (WT) controls. Interestingly, stimulation with norepinephrine resulted in an increased propensity for DADs, which was suppressed by flecainide. AP recordings revealed prolonged APD also suggesting a clinical overlap between TECRL mutation-positive individuals with features of both CPVT and LQTS.[102] At present, the mechanisms by which loss of TECRL function alters SR Ca^{2+}-handling or ionic currents resulting in prolonged APD remain unknown.

CALM1-encoded calmodulin type-1 (CPVT-4)—A fourth subtype of CPVT (CPVT-4) is caused by inherited mutations in *CALM1*-encoded calmodulin (CaM). The locus for this variant, 14q31-q31, was initially found by linkage analysis in a large, multigenerational Swedish family.[103]. Family members demonstrated multiple episodes of syncope and sudden death, particularly with exercise and exertion. On clinical evaluation, affected individuals demonstrated ventricular ectopy and evidence of VT/VF that was suppressed by β-blockers. The genetic haplotype was inherited in an autosomal dominant fashion and was completely penetrant. Subsequent genetic analysis of the approximately 70 known genes within the locus demonstrated a heterozygous CaM-N53I mutation that segregated with incidence of disease within the family. A second mutation, CaM-N97S, was identified in an unrelated proband from Iraq who was diagnosed with CPVT and was negative for mutations in *RYR2*.

Calmodulin is a ubiquitously expressed Ca^{2+}-sensitive signaling molecule which is found in all eukaryotic cells.[104]. There are three *CAM* genes in humans, *CALM1*, *CALM2*, and *CALM3* which all encode a single protein – CaM. CaM is a relatively small, 148 amino acid alpha-helical protein with four classical Ca^{2+} binding EF hands that each bind to a single Ca^{2+} cation. This direct Ca^{2+}-binding property allows conformational shifts in the N- and C-terminal domains of the protein which mediate a variety of interactions with a large number of intracellular binding targets.[105]. A dumbbell-shaped molecule, CaM can sense both local and global Ca^{2+} levels, which allows for exquisite sensitivity to a variety of Ca^{2+}-signaling events with downstream regulation of a number of Ca^{2+}-handling proteins.[106] Within the heart, CaM plays a key role in EC coupling and is critical for the SR Ca^{2+} release and subsequent Ca^{2+} re-uptake into the SR. The LTCC and RyR2 are both important binding partners of CaM.[107] Ca^{2+} entering the cardiomyocyte via LTCCs binds to CaM which, in turn, binds to the C-terminal IQ domain of the Cav1.2 channel α pore subunit (α_{1C}) of LTCC. This process allows Cav1.2 channels to cluster and interact with each other, allowing for sufficient Ca^{2+} entry to initiate EC coupling.[108] CaM also binds to RyR2, and binding of CaM reduces the open probability of RyR2. Conversely, impaired binding of CaM to RyR2 due to a mutated binding domain on RyR2 leads to a variety of cardiac pathologies.[109].

In vitro experiments have revealed that mutations in the gene encoding CaM compromise Ca^{2+}-binding and result in an aberrant interaction with the CaM-binding domain of

RyR2.[103] Subsequent studies revealed that the CaM-N97S mutation in the C-domain reduced Ca^{2+}-binding affinity of the C-domain and impaired binding to RyR2 at low Ca^{2+} concentrations, which was predicted to lead to an increased RyR2 open state. This impaired inhibitory gating regulation was confirmed by subsequent studies of RyR2 single channel recordings in the presence of mutant CaM and functionally resulted in an increased susceptibility for RyR2-mediated store overload-induced Ca^{2+} release (SOICR).[110, 111] In contrast, the CaM-N53I variant, which localized to the opposing N-domain, demonstrated a small yet significant increase in the Ca^{2+}-saturation of the C-domain with an alteration to RyR2 binding affinity. These findings demonstrated that mutations in *CALM1* are associated with CPVT through two distinct mechanisms of RyR2 dysregulation and support a model whereby the Ca^{2+}-saturated C-lobe is constitutively bound to RyR2 while the N-lobe senses fluctuations in cellular Ca^{2+}.[111]

TRDN-encoded triadin (CPVT-5)—Finally, a fifth subtype of CPVT (CPVT-5) is caused by mutations in *TRDN*-encoded TRDN. Mutations in *TRDN* were first identified by candidate gene approach, and a small number of probands were identified with either homozygous loss-of-function (LOF) mutations or compound heterozygous mutations. For example, a homozygous frame-shift mutation, TRDN-D18Afs*13, was noted in a proband with cardiac arrest at age of 2 years who was found to have polymorphic VT.[112] A second independent proband hosted two mutations, TRDN-Q205X and –T59R, and demonstrated proximal muscle weakness, syncope with exertion and bidirectional ventricular ectopy.[112] Thus, *TRDN* mutations can cause CPVT in an autosomal-dominant manner.

As discussed above, TRDN is a transmembrane protein on the SR that forms a macromolecular complex with RyR2, CASQ2, and JCTN.[113] TRDN is a multiprotein family arising from alternative splicing of a single *TRDN* gene. Two isoforms are exclusively expressed in skeletal muscle, whereas a third isoform (also known as Trisk 32 or CT1) is expressed mainly in cardiac muscle.[114] Interestingly, all three *TRDN* mutations localized to a region of the protein that is common to all isoforms, including skeletal muscle isoforms.[112] The link between *TRDN* mutations and skeletal myopathy remains unknown. *In vitro* functional analysis of the TRDN-T59R mutation in non-muscle COS-7 cells demonstrated intracellular retention and degradation of the mutation protein. Further, viral transduction of TRDN-T59R mutant protein into Trdn−/− mice demonstrated no expression of the protein by immunofluorescence of isolated cardiomyocytes.[112] Thus, functionally CPVT-associated mutations lead to a severe TRDN function in cardiomyocytes. Electron microscopy studies of cardiomyocytes from Trdn−/− mice revealed fragmentation and overall reduction in contacts between the junctional SR and T-tubules.[115] The function of CRU channels was impaired with reduced negative feedback of SR Ca^{2+} release on $I_{Ca,L}$. This uninhibited sarcolemmal Ca^{2+} influx via $I_{Ca,L}$ likely caused SR Ca^{2+} overload leading to spontaneous SR Ca^{2+}-release events upon βAR stimulation.

Congenital long QT syndrome (LQTS)

Congenital long QT syndrome (LQTS) refers to a distinct group of cardiac channelopathies characterized by delayed cardiac repolarization, which places affected individuals at risk for syncope, seizures, and SCD. A relatively common arrhythmia syndrome, affecting as many

as 1 in 2,500 persons, this delay in cardiac repolarization occurs in the absence of an underlying syndrome or structural heart disease.[116, 117] Approximately 75% of LQTS cases are due to mutations in three genes: *KCNQ1*-encoded I_{Ks} potassium channel (Kv7.1, LQTS-1), *KCNH2*-encoded I_{Kr} potassium channel (Kv11.1, LQTS-2), and *SCN5A*-encoded I_{Na} sodium channel (NaV1.5, LQTS-3).[118] These ion channels play key roles in the cardiac AP and genetic defects in these channels delay repolarization. Several channel interacting proteins, such as *ANK2*-encoded ankyrin B (LQTS-4), *KCNE1*-encoded min-K (LQTS-5), and *KCNE2*-encoded min-K related protein 1 (LQTS-6), among others, interact with these major channels and have been implicated as rare causes of LQTS.[119, 120] To date, hundreds of mutations have been identified in 17 LQTS-susceptibility genes (Table 2). In addition, large population-based GWAS analysis exploring common genetic variants associated with QT prolongation have identified a number of loci which encode Ca^{2+}-signaling proteins that were associated with longer QT durations.[121] While the majority of the accepted LQTS genes encode proteins which govern the flux of Na^+ and K^+ about the sarcolemma, there is mounting evidence that Ca^{2+} fluxes and intracellular Ca^{2+} signaling are associated with prolonged cardiac repolarization and LQTS.

***CACNA1C*-encoded L-type calcium channel (LQTS-8)—**The *CACNA1C* gene encodes the Cav1.2 (β_{1C}) channel subunit of the LTCC, a macromolecular channel complex responsible for $I_{Ca,L}$ and EC coupling.[3] The Cav1.2 protein is comprised of 4 homologous domains (DI through DIV) that are connected by intracellular linker regions (I-II, II-III, and III-IV loops) and 6 transmembrane segments (S1 through S6).[122] Mutations in *CACNA1C* have been associated with a number of human diseases that have cardiac manifestations. Classically, mutations in *CACNA1C* have been associated with Timothy syndrome (TS) – a disease characterized by extreme QT interval prolongation, syndactyly, neurodevelopmental delay, and SCD predisposition.[123–126] Expansion of clinical genetic testing has identified a number of *CACNA1C* mutations in individuals demonstrating only cardiac abnormalities (QT prolongation, structural heart disease, and cardiomyopathy), without extracardiac abnormalities, so-called cardiac-only Timothy syndrome (COTS).[127] Individuals with only QT prolongation, and a diagnosis of LQTS, have been identified in a large number of independent cohorts.

Many *CACNA1C* mutations have been characterized *in vitro* through heterologous expression in cell lines such as HEK293 and TSA201 cells, and demonstrate either increased peak $I_{Ca,L}$, decreased current density with increased window current, or negative activation/positive inactivation shifts.[128] Experimental and modeling studies have demonstrated that mutant *CACNA1C* can lead to enhanced $I_{Ca,L}$, and DAD-mediated triggered activity.[129] In addition, they can steepen the APD restitution curve, disrupt rate-dependent cardiac excitation dynamics, and promote the development of alternans.[130] Finally, *CACNA1C* mutations can amplify dispersion of repolarization across the tissue, which produces T-wave alternans and T-wave inversion on the ECG.[130, 131]

While the overall functional impact of these mutations is the prolongation of phase-2 of the AP causing delayed repolarization, there does not appear to be a clear mechanistic difference between the *CACNA1C* mutations that lead to TS, COTS, or LQTS. Indeed, this is reflected in the recent identification of a CACNA1C-I1166T in a proband with TS, and independently

identified CACNA1C-I1166V mutation, localizing to the identical residue, in a patient with LQTS.[132, 133] Given the lack of robust mechanistic studies, it remains unclear how a near-identical genetic substrate can lead to variable expressivity and severity of a disease phenotype. It is likely that genetic modifiers contribute to the differential phenotype manifestations. Additional mechanistic studies, perhaps utilizing iPSC-CM derived from TS, COTS, and LQTS patients with *CACNA1C* mutations may yield insight into genomic, epigenomic, molecular, and biophysical changes that are specific to each disease presentation.

***CALM1*, *2*, and *3*-encoded calmodulin 1, 2, and 3 (LQTS-14-16)—**In 2013, the first mutations in the *CALM1* and *CALM2* genes were associated with LQTS.[134] Two unrelated infant probands were described with a severe phenotype of recurrent cardiac arrests with markedly elevated QTc intervals. They were each found to host a heterozygous mutation – CaM-D130G and CaM-D96V mutations, respectively.[134] Subsequent validation genotyping in a cohort of LQTS patients yielded an unrelated proband with CaM-D130G and a second subject with CaM-F142L. These mutations were all found to localize either within, or immediately adjacent to, the third and fourth EF hand domains of the C-terminal lobe, resulting in impaired Ca^{2+}-binding of the domain.[134] Interestingly, subsequent biochemical investigations have elucidated two distinct mechanisms of CaM and RyR2 dysregulation. CaM-D130G and -D96V both impaired CaM-dependent inhibition of RyR2, resulting in an increased open state when single channels were recorded and an increased propensity for SOICR.[110] In contrast, while CaM-F142L demonstrated reduced Ca^{2+} binding, it was unexpectedly found to enhance CaM-dependent gating inhibition of RyR2 and related RyR2-mediated SOICR. Specialized thermodynamic and NMR spectral analysis of the interaction between CaM-F142L and the reciprocal binding domain of RyR2 demonstrated unique alterations in the protein-protein interface suggesting that the mutation does not disrupt the negative regulatory role of CaM despite an impaired ability to bind free Ca^{2+}.[110] In addition to mutations identified in *CALM1* and *2*, the first reports of LQTS-associated mutations in *CALM3* have been recently reported. Specifically, a CaM-D130G mutation was identified in a neonate with a profoundly elevated QTc interval.[135] To date, mutations in *CALM3* have not been widely identified and there have been no robust mechanistic studies to evaluate the role of CaM in LQTS. Taken together, these studies identify divergent mechanisms of disease pathogenesis that can, nonetheless, result in altered RyR2 inhibition by CaM.

As previously described, the loss of RyR2 gating inhibition is classically associated with the development of CPVT, and the link between these mutations and LQTS remains unexplored. One explanation for this dichotomy is that there are additional molecular effects to impaired CaM activity, such as increased $I_{Ca,L}$, which can prolong the APD. This possibility is supported by early studies in guinea pig cardiomyocytes which demonstrate reduced CaM-dependent inactivation of $I_{Ca,L}$ with expression of LQTS-associated *CAM* mutations. In addition, LQTS-associated *CAM* mutations result in electrical alternans in a high dispersed manner across and within cells consistent with the electrical remodeling observed in canonical LQTS-associated mutations.[136] Given the clear role of these mutations on RyR2 gating, it is likely that there is significant molecular overlap between the LQTS- and CPVT-

associated mutations. However, the effect of CPVT-associated mutations in other sarcolemmal ionic currents that shape APD and cardiac repolarization are largely unexplored, although the Nav1.5 and delayed rectifying I_K currents are strong candidates.

Recently, the first attempts to derive patient-specific therapies to mitigate the abnormally prolonged repolarization have been reported. In 2017, human iPSC-CMs were derived from a subject who was diagnosed with LQTS shortly after birth following a cardiac arrest with a markedly elevated QTc of 740 who hosted a CaM-D130G mutation.[137] Human iPSC-CMs derived from dermal fibroblasts demonstrated prolonged APD and larger Ca^{2+} transients with slower rise and decay kinetics when compared to WT iPSCs from an unrelated ostensibly healthy donor.[138] Further, CaM-D130G imparted a significant decrease to CaM-dependent inactivation of the LTCC. The authors utilized CRISPR-mediate interference of the transcription of *CALM2,* which specifically reduced expression of the mutant protein without altering expression of either *CALM1* or *CALM3*. This selective expression inhibition rescued the prolonged APD in iPSC-derived cardiomyocytes.[138] While this study represents a major step forward in gene therapy-approaches to altering monogenic disease expression, translating this technique to an *in vivo* model of arrhythmia remains an active and challenging area of exploration.

***TRDN*-encoded triadin (LQTS-17)—**The most recent gene associated with LQTS is *TRDN*-encoded TRDN, which has been previously also linked to CPVT-5. Identified following WES of probands negative for the known LQTS-associated genes, a handful of *TRDN* null variants were identified. As with CPVT-5, each mutation-positive proband demonstrated either homozygous inheritance of LOF allele or a compound heterozygous mutation with a LOF allele. [112, 139] Both entities clinically manifest as SCD with either QT prolongation (LQTS-17) or signs of ventricular ectopy in the absence of QT prolongation (CPVT-5) diagnosed at an early age. This combination of clinical findings, in addition to the skeletal muscle weakness occasionally noted with CPVT-5, and the *TRDN* genetic substrate has been labeled the so-called triadin knockout syndrome. To date, there have been no mechanistic studies involving LQTS-associated *TRDN* mutations, and while Trdn−/− mice have a known propensity for arrhythmogenesis with βAR stimulation, QT prolongation has not been detected.[115, 140] Given the previously described possibility that Trdn−/− mice likely demonstrate reduced negative feedback of RyR2-mediated SR Ca^{2+}-inhibition of $I_{Ca,L}$, an interesting possibility is that the increased Cav1.2 current might lead to APD prolongation. This would be an indirect mechanism of QT prolongation that is analogous to the *CACNA1C* mutations described in LQTS-8 which produce an increased $I_{Ca,L}$ current. Further extensive studies are needed to delineate these hypotheses.

Idiopathic Ventricular Fibrillation (IVF)

IVF is a genetic disease characterized by a documented VF event that is otherwise unexplained. Comprising approximately 1% of out-of-hospital cardiac arrest survivors presenting with a shockable rhythm, IVF can often be challenging to diagnosis.[141, 142] Further, in the setting of a normal ECG, the affected status of an individual can only be known following an arrhythmic event, which makes genetic studies challenging. Traditionally associated with mutations in the *SCN5A*-encoded Nav1.5, the first IVF-

associated mutations were often described in sporadic cases presenting with VF and had significant clinical overlap with a group of Nav1.5-mediated channelopathies known as Brugada syndrome (BrS).[143, 144] New genetic testing platforms have allowed for the identification of other IVF genes implicated in families with the arrhythmia (Figure 5) and recent advances in WES have identified the first genes encoding Ca^{2+}-handling proteins in children with IVF.

In 2014, a family with a history of VF and SCD with normal ECG and echocardiograms was subjected to WES after kindred were found to be genotype-negative for the major LQTS, CPVT, and arrhythmogenic right ventricular cardiomyopathy (ARVC)-associated genes. This identified a CALM1-F90L mutation in a proband who experienced out-of-hospital arrest due to VF at age 16 with no clinical evidence of LQTS, CPVT, cardiomyopathy, or other SCD-predisposing etiology.[145] Subsequent functional evaluation of the CALM1-F90L mutation demonstrated impaired CaM stability and impaired Ca^{2+} binding cooperativity.[109] It was concluded that the F90L mutation likely perturbs the position of two Ca^{2+} EF hands within the C-lobe relative to each. As a result, the ability of the first occupied site to induce a favorable conformational shift in the second, which is needed to facilitate Ca^{2+}-binding, is impaired. The authors concluded that this creates a relatively insensitive CaM protein which is not responsive over small changes in Ca^{2+} concentration.[109]

While the impact of the F90L on the function of CaM is known, the ultimate effect of this perturbation on RyR2 gating or other Ca^{2+}-handling proteins are still unknown. While there has been some incremental progress in identifying the genetic and molecular etiology of IVF, mutations remain rare and IVF remains enigmatic as a disease entity. As with the development of CPVT and LQTS, one possibility is that altered CaM function associated with IVF selectively impairs some ion channels while leaving other channels unaltered. A tempting target is Nav1.5, which contains a number of IVF-associated mutations. *SCN5A*-associated IVF and BrS mutations demonstrate a diverse array of biophysical effects in heterologous cell line over-expression models. For example, some *SCN5A* IVF/BrS mutations create depolarizing shifts in channel inactivation while others create hyperpolarization shifts in both activation and inactivation, all with the ultimate effect of loss-of-function effect on Nav1.5 and VF predisposition.[143, 146] It is possible that IVF-associated *CAM* mutations results in loss of Nav1.5 depolarizing current and dispersion of excitability – a known molecular substrate for reentry-mediated VF. This possibility is supported by structural evidence that CaM directly binds Nav1.5 and is a critical player in channel inactivation and permitting channel activation. However, a direct link between CaM mutations and VF has not been clearly demonstrated.[105] Ultimately, subsequent studies are needed to link *CAM* mutations to I_{Na} current and a reentry substrate in IVF.

Hypertrophic Cardiomyopathy (HCM)

HCM is an inherited cardiac disorder characterized by asymmetrical hypertrophy of the heart, with a prevalence of 1 in 500.[147] This disease represents the most common cause of arrhythmogenic SCD in the young, particularly in young athletes.[148] HCM is not only associated with lethal ventricular arrhythmias, but also with AF.[149, 150] Since the association of the first mutation gene with HCM, *MYH7*-encoded β-myosin heavy chain, multiple

studies have determined that the majority of HCM cases are due to mutations in genes encoding components of the cardiac sarcomere.[151–153] While the cardiac myofilaments are the major molecular cause of HCM, Ca^{2+} dysregulation plays a significant role in the pathologic remodeling and hypertrophy. Further, abnormal Ca^{2+}-signaling and the myofilament sensitivity to Ca^{2+}, are both known triggers for ventricular arrhythmias. Sarcomeric HCM genes are divided into sub-groups based location of the encoded protein in the cardiac sarcomere consisting of the thick, intermediate, and thin myofilaments. Mutations in genes encoding the thick myofilament (*MYH7*-encoded beta myosin heavy chain, *MYL2*-encoded regulatory myosin light chain, and *MYL3*-encoded essential myosin light chain), the intermediate myofilament (*MYPBC3*-encoded cardiac myosin binding protein C), and the thin filament proteins (*ACTC*-encoded actin, *TPM1*-encoded alpha-tropomyosin, *TNNT2*-encoded cardiac troponin T (TnT), *TNNI3*-encoded cardiac troponin I (TnI), and *TNNC1*-encoded cardiac troponin C (TnC)) have been linked with development of HCM.[154–160] Mechanisms of sarcomeric HCM pathogenesis have been extensively reviewed.[161, 162]

Arrhythmia predisposition in sarcomeric HCM

Early in the exploration of the sarcomeric gene-association with HCM, it was proposed that the arrhythmia burden, manifest in SCD, might be higher with certain mutations. For example, early studies identified individuals in large families of HCM hosting either the MYH7-R403Q or -R453C missense mutations with increased sudden deaths compared to those hosting a -V606M mutation.[163] Further, early genotype-phenotype studies of *TNNT2* suggested an association with decreased life expectancy and a high incidence of SCD despite minimal cardiac hypertrophy.[164] These studies proposed that individual mutations, or mutations in specific genetic loci, may predispose to lethal arrhythmic events in HCM. As the field has matured, these associations were not universally observed, and there is significant heterogeneity in the expression and penetrance of sarcomeric HCM disease.[165–168] This controversy has been previously reviewed.[169, 170] Overall, these genotype-phenotype correlations did not have mechanistic support for the arrhythmia burden observed in some cases; however, a growing body of evidence suggests myofilament Ca^{2+}-sensitivity as a major arrhythmic mechanism which is independent of gene mutation. As a molecular unit, the troponin complex and thin filament proteins are responsible for sensing intracellular Ca^{2+} fluctuations and triggering sarcomeric contraction.[171] While many myofilament proteins have been linked to HCM arrhythmogenesis, alterations in Ca^{2+}-sensitivity of the components of the thin filament have been most clearly linked with potentially fatal ventricular arrhythmias.[172] This is detailed below.

While HCM carries an increased risk of lethal ventricular arrhythmias,[173] atrial fibrillation (AF) is found commonly with a frequency of 20–25% of all patients with HCM.[174] The hemodynamic mechanism of this may be related to atrial dilation secondary to elevated left ventricular filling pressures resulting in left atrial dilation; however, the cellular mechanism of this is unexplored among sarcomeric HCM. Further, while there have been some suggestions the sarcomeric mutations may predispose individuals for early and more severe AF,[175, 176] there have not been conclusive studies linking specific genotype to AF predisposition.[150]

***TNNT2*-encoded cardiac troponin T and *TNNC1*-encoded cardiac troponin C—** Troponins are the Ca^{2+}-sensing molecule of the myofilament. Following CICR, free Ca^{2+} binds TnC which increases its binding affinity for TnI, pulling the TnI inhibitory domain away from its binding site on actin through its interaction with the molecular linker TnT.[177] This permits the troponin-tropomyosin complex to move further into the actin groove fully exposing the myosin binding sites on actin. Active actin-myosin cross-bridging then occurs and contraction begins.[177]

Traditionally, mutations in *TNNT2* were believed to be more arrhythmogenic compared to other genetic subtypes of HCM.[178] While this belief has been called into question recently,[169] a significant body of evidence has linked HCM-associated *TNNT2* mutations with the development of fatal arrhythmias in the absence of other known predictors of arrhythmia predisposition such as significant hypertrophy or fibrosis. A number of TnT mutations have been described that nearly universally increase Ca^{2+} sensitivity, and thus Ca^{2+}-binding of TnT and the sarcomeric thin filament. It is believed that TnT serves as a molecular sink for dynamic Ca^{2+}-buffering, and that increased Ca^{2+}-sensitivity may lead to altered Ca^{2+}-transient dynamics. Overall, the degree of arrhythmia susceptibility appears to be directly correlated to the degree of increased Ca^{2+} sensitivity.[179]

A mouse model of HCM (transgenic over-expression of I79N mutant TnT) exhibits increased cardiac contractility with reduced diastolic relaxation in the absence of significant fibrosis, as well as increased myofilament Ca^{2+} sensitivity.[180] This increased Ca^{2+}-sensitivity was associated with increased diastolic Ca^{2+} levels and intracellular Ca^{2+} overload in isolated cardiomyocyte studies.[181] Further, TnT-I79N was associated with decreased Ca^{2+}-transient amplitudes in the face of elevated resting Ca^{2+} levels which caused ventricular ectopy and VT.[182] While the precise mechanism has not been clarified, the increased TnT Ca^{2+} sensitivity may lead to DAD-mediated VT resulting from reduced myofilament Ca^{2+} buffering or could cause reentrant arrhythmia through a still undefined mechanism. The first option is supported in other models of increased thin filament Ca^{2+} sensitivity. The transgenic expression of fetal slow skeletal troponin I (ssTnI) in place of TnI increased Ca^{2+} sensitivity in a manner analogous to the electrical remodeling found in pathologic hypertrophy.[183] In this model, constitutive increase in Ca^{2+} sensitivity is associated with increased expression of NCX which might result in increased I_{NCX} current to maintain Ca^{2+} homeostasis during diastole when SERCA2a is also reduced.[184] Interestingly, this observation was noted in younger but not in older mice, reflecting the early age of onset of arrhythmias in *TNNT2*-positive subjects. The reentry hypothesis is supported by evidence that *TNNI3* mutations can increase spatial dispersion of activation times across the myocardium, thereby promoting reentry. For example, *TNNT2* mutations, including TnT-179N, have been shown to associate with a short effective refractory period along with beat-to-beat variability in APD with increased spatial dispersion of conduction velocity.[179] Additional studies are required to directly prove two suggested hypotheses and delineate the underlying arrhythmogenic mechanisms associated with *TNNT2* mutations.

Mutations in *TNNC1*, a rare cause of HCM, have been also linked with a predisposition to fatal arrhythmias. A TnC-A31S mutation was identified in 3-year-old boy who had HCM and an out-of-hospital VF event. Despite being on β-blocker therapy, he had multiple

breakthrough VF events with appropriate ICD discharged. This mutation is located within the inactive Ca^{2+}-binding domain of TnC. When reconstituted in skinned porcine cardiac fibers, this resulted in increased Ca^{2+}-sensitivity of both TnC and the thin filament compared to WT.[185] Should future studies confirm the presence of increased cellular Ca^{2+} levels, this also raises the possibility of either a DAD-mediated trigger or formation of an arrhythmogenic substrate for reentry. In addition, while rare, identification and characterization of human mutations affecting other thin filament components will add additional mechanistic understanding to this process.

***JPH2*-encoded junctophilin type 2—**A small subset of patients without mutations in sarcomeric genes host a genetic mutation in genes encoding Ca^{2+}-handling proteins, and some have been linked with a predisposition to arrhythmia.[186] *JPH2*-encoded JPH2, is a member of the junctophilin family of proteins which plays a critical role in maintaining the JMC in excitable cells, including striated muscle.[6, 7] JPH2 is the major family member found in the heart and spans the JMC, tethering the SR to the sarcolemma creating a fixed cardiac dyad distance as well as serving a key role in negatively regulating RyR2 opening (Figure 1B).[7, 187] Reflective of the critical role that this protein plays in maintain CICR as well as Ca^{2+} homeostasis by RyR2 gating regulation, JPH2 plays a prominent role in cardiomyopathy development, HF progression, and development of EC coupling in the immature myocyte.[188–191] These diverse roles have been previously reviewed.[192, 193] An emerging role of JPH2 is the development of Ca^{2+}-mediated arrhythmias, in particular congenital AF. While the vast majority of AF is acquired, reviewed in detail below, a specific mutation (E169K) in JPH2 was linked with AF development in a small family with HCM.[194] Expression of JPH2-E169K in mice demonstrated a higher incidence of pacing-induced AF with increased SR Ca^{2+} leak and propensity of ectopic Ca^{2+} transients following rapid pacing.[194] This was associated with increased RyR2-mediated SR Ca^{2+} leak due to loss of direct binding between RyR2 and JPH2.[189, 194] This contributed to increased diastolic Ca^{2+}, increased NCX activity, and a predisposition to DADs. Additional studies are needed to more thoroughly dissect the molecular underpinnings of JPH2-mediated atrial electrical remodeling.

***CASQ2*-encoded calsequestrin 2 and *CALR3*-encoded calreticulin type 3—**Rare mutations in other members of the JMC and RyR2 macromolecular complex have been linked to HCM. Genetic interrogation of an Australian cohort of 252 unrelated individuals with HCM revealed a single mutation, D63E in *CASQ2* as well as 2 mutations in the *CALR3* gene (CALR3-R73Q and -K82R) that were not identified in the ostensibly healthy control population.[195] To our knowledge, these are the only mutations described in these genes among individuals with cardiomyopathy. The CASQ2-D633 was found in compound heterozygosity with two *MYBPC3* mutations, which decrease the likelihood of a truly causative biomarker. Conversely, the 2 *CALR3* mutations were found in genotype-negative individuals. CALR is a Ca^{2+}-binding chaperone in the sarcoplasmic/endoplasmic reticulum, where it buffers Ca^{2+} and plays an important role in the quality control of intracellular secretory pathway processes.[196] CALR has two isoforms, and little is known about the expression levels of CALR3 in myocardial tissue. The functional implications of the *CALR3* variants are presently unknown. In embryonic stem cell knockout model, CALR3 deficiency

compromised the nuclear pore complex and disrupted the nuclear import of the cardiac transcription factor MEF2C in a Ca^{2+}-dependent manner.[186, 197]

Arrhythmogenic Cardiomyopathy (ARVC)

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/C), also referred to as arrhythmogenic cardiomyopathy, is a relatively rare type primary myocardial disease characterized by fibro-fatty replacement of myocardial tissue, cardiac arrhythmias, and an increased risk of SCD. This disease process has been recently reviewed.[198] Traditionally, ARVC is considered a disease of the cardiac desmosome, whereby mutations in components of this cell-cell adhesion structure are commonly identified in individuals with disease.[199, 200] There is some evidence that *RYR2* mutations may be a rare cause of ARVC or are present with a prevalence that is significantly higher than rare *RYR2* variants in a control population.[201, 202] These clinical observations suggests that *RYR2* variants play a role in the genetic basis of traditional ARVC as either disease-causing mutations or as a modifier susceptibility allele. In a mouse model of an ARVC-linked *RYR2* variant, a reduced right ventricular end-diastolic volume was observed, but pathognomic fibrofatty infiltration or structural abnormalities seen in ARVC patients were absent.[59] Despite the possible link between RyR2 and ARVC, there currently is insufficient evidence to implicate primary defects in Ca^{2+}-signaling in the pathogenesis of this disorder.[203]

Dilated cardiomyopathy (DCM)

Familial dilated cardiomyopathy (DCM) is a genetic heart muscle disease characterized by progressive dilation and dysfunction of the left or both ventricles. Mutations in over 30 genes can cause congenital DCM, and most of these genes encode proteins that are part of the sarcomere or are structural proteins needed to conduct mechanical force in the cardiomyocyte.[204] The remaining genes encode proteins that play various roles within cardiomyocytes to ensure proper contractile function. Various studies suggest that mutations in sarcomeric genes[205–207] as well as non-sarcomeric genes[208, 209] can alter Ca^{2+} homeostasis, although the affected proteins are not directly involved in Ca^{2+}-handling. On the other hand, there is a clear role of defective Ca^{2+}-handling in DCM pathogenesis in patients with inherited mutations in phospholamban (PLN) and histidine-rich Ca^{2+}-binding protein (HRC).

***PLN*-encoded phospholamban—**Rare mutations and polymorphisms localizing to *PLN* have been linked to patients with inherited cardiomyopathy. In a large family with multiple generations of cardiomyopathy, kindred homozygous for a PLN-L39X nonsense (early stop) mutation developed DCM and HF requiring cardiac transplantation as adolescents.[210] Interestingly, individuals heterozygous for this mutation tended to demonstrate HCM. This raises the possibility of a dose-dependent effect with loss of *PLN* expression. In addition, multiple small genotyping studies identified a handful of heterozygous *PLN* mutations in individuals that were missense.[211, 212] Moreover, *PLN* mutations have been identified in individuals with HCM exclusively. These include a handful rare *PLN* promoter have been identified in multiple independent cohorts.[213–215] Overall, it appears that mutations in *PLN* are a rare cause of both DCM and HCM accounting for less than 1% of all individuals with disease.[213]

As previously discussed, myocyte relaxation during diastole is an active process mediated by ATP-expending pumping of cytosolic Ca^{2+} into the SR lumen via SERCA2a, which is negatively regulated by PLN. Moreover, PLN inhibitory action can be reduced by PKA and CaMKII-mediate phosphorylation.[216, 217] Since the discovery of cardiomyopathy-associated mutations, several mouse models have been made expressing putatively pathogenic mutations. For example, transgenic overexpression of the PLN-R14del mutation, initially identified in a large family of DCM, lead to cardiac dilation, myocyte disarray, fibrosis and early death in a mouse model.[211] *In vitro* studies of PLN-R14del expressed in HEK293 cells demonstrated super-inhibition of Ca^{2+} affinity for SERCA2a that was not relieved by PKA phosphorylation. While the precise mechanism of *PLN*-mediated DCM remains unclear, it is possible that chronic suppression of SERCA2a activity leads to increased cytosolic Ca^{2+} and a substrate for DAD arrhythmogenesis and, perhaps concurrently, pathologic myocardial remodeling resulting in HF. This possibility is supported by recent work exploring a PLN-R25C mutation. Originally identified by WES of a DCM family with significant ventricular arrhythmias requiring ICD placement, this mutation caused super-inhibition of SERCA2a when virally over-expressed in adult cardiomyocytes.[218] This resulted in decreased SR Ca^{2+} content and reduced Ca^{2+} transient amplitude which is consistent with the loss of systolic function observed in DCM. Further, increased Ca^{2+} spark frequency and spontaneous Ca^{2+} waves were seen, suggesting DAD-type arrhythmia susceptibility.[218] While the mechanism of increased SR Ca^{2+} leak is unknown, it is possible that CaMKII activity is increased in the setting of elevated myocyte Ca^{2+} levels. Additional studies specifically exploring the interaction between SERCA2a function and RyR2 gating are needed to clarify this relationship.

***HRC*-encoded histidine-rich calcium-binding protein—**Candidate gene-based genetic interrogation of a cohort of DCM patients for *HRC*-encoded HRC identified a S96A polymorphism that was statistically associated with the development of ventricular arrhythmias. Presence of the minor allele variant conferred a hazard ratio of 4.2 for VT or VF among individuals with DCM.[219] HRC is part of the SERCA2a macromolecular complex and serves as a regulator of SR Ca^{2+} reuptake (Figure 4). It has been shown to bind the SR luminal side of SERCA2a and interact with TRDN.[32, 33] Subsequent studies utilizing adenoviral overexpression in rat ventricular myocytes demonstrated reduced SR store Ca^{2+} reuptake and increased Ca^{2+} sparks with HRC-S96A expression compared to WT.[220] Interestingly, this phenotype was exacerbated following myocardial ischemia and resulted in spontaneous Ca^{2+} waves.[220] This suggested an arrhythmia susceptibility allele that may alter RyR2 gating function in the setting of ischemic stress. While *in vivo* studies are needed to validate these observations, the findings together suggest a relatively common variant that may be clinically silent until an acquired myocardial stress or injury. Further, these findings suggest a molecular mechanism of cross-talk between SR reuptake via SERCA2a and SR release via RyR2. Whether this association is direct through common binding partners, indirect through signal transduction molecules, or a combination of both, remains unknown.

CALCIUM-DEPENDENT ACQUIRED ARRHYTHMIAS

While a number of arrhythmias can be caused by heritable mutations in cardiac ion channels and channel interacting proteins, Ca^{2+}-mediated arrhythmias can also develop in the setting of acquired diseases of the myocardium. These common types of arrhythmias include atrial fibrillation (AF) and ventricular tachyarrhythmias encountered in patients with structural heart disease.[75, 221] In this review, we will not discuss arrhythmias that occur in conjunction with structural heart disease.

Heart Failure (HF)

Heart failure is a clinical diagnosis, which is defined as any abnormality in cardiac structure or function which results in failure of the heart to meet the metabolic demands of the body. Affecting millions of people worldwide, an estimated 1 in 5 people will develop heart failure during their lifetime, making it one of the most deadly, morbid, and expensive diseases known.[222] While gains have been made in reducing mortality, there is recent evidence that these gains have plateaued and that global burden of HF remains high.[223, 224] Given this, there have been rapid advances in the pharmacologic management of HF, as well as guidelines shifts for recommended therapies, which target a growing number of molecular mechanisms.[225] Pathologic alterations in cardiomyocyte Ca^{2+} cycling have emerged as a prominent component of the molecular dysfunction that occurs in HF. Understanding these mechanisms has been central to the development of recent novel therapies. These topics have been heavily reviewed.[226–228] Further, a substantial body of evidence exists linking these pathologic alterations in Ca^{2+} cycling to arrhythmic predisposition during HF remodeling. These arrhythmias are the cause of a significant proportion of SCD which occurs during HF.[229, 230]. Just as HF is a complex and varied disease, the arrhythmic substrate from aberrant Ca^{2+} signaling is a broad subject and has been the topic of multiple comprehensive reviews.[15, 231, 232]

Atrial fibrillation (AF)

AF represents the most common type of cardiac arrhythmia observed in the general population.[233] This disease often progresses from a more intermittent form (paroxysmal AF; pAF) to persistent (chronic) AF (cAF) which lasts for more than 7 days at a time.[36, 221] Numerous factors can promote the occurrence of AF, including genetic determinants (Figure 6), extra-cardiac factors (e.g., sleep apnea, obesity, hypertension, autonomic imbalance), as well as remodeling of the cardiac tissue.[36, 234] In this section, we will focus on the potential involvement of Ca^{2+} in the development of AF. The primary arrhythmia mechanisms contributing to AF are focal ectopic firing and reentrant activity (Figure 7).

Ca^{2+}-dependent triggered activity in AF—Experimental studies in animal AF models and atrial samples from AF patients revealed that abnormal atrial Ca^{2+}-signaling likely plays a role in AF pathophysiology by contributing to afterdepolarization-mediated triggered activity, conduction block, and Ca^{2+}-driven subcellular alternans.[36, 235] Cellular DAD-mediated triggered activity was demonstrated in atrial myocytes from patients with pAF (Figure 7A).[236] These patients were in sinus rhythm at the time of tissue collection for weeks, thus excluding confounding effects of high atrial rate-induced atrial remodeling.

Several factors contribute to the increased incidence of spontaneous SR Ca^{2+} release events, including increased SR Ca^{2+} load and enhanced RyR2-mediated SR Ca^{2+} release. The SR Ca^{2+} stores are overloaded due to increased SERCA2a activity secondary to PLN phosphorylation resulting in inactivation of the inhibitory protein.[236] Increased SR Ca^{2+} leak was caused by increased RyR2 protein levels and RyR2 activity levels, whereas RyR2 phosphorylation levels were unaltered.[194, 237] Enhanced RyR2 protein expression during pAF appears to be caused in part by a reduced expression of the microRNA cluster miR-106b-25, which enhances post-transcriptional regulation of RyR2.[238] Consistent with these data is the finding that miR-106b-25 deficient mice are more susceptible to pacing-induced AF, atrial ectopy, and increased SR Ca^{2+} release events.[238] Recent transcriptomic analyses suggest that there may be additional alterations in miRNA and mRNA which have not been fully explored in patients with pAF.[239] Taken all of these studies together, it is clear that additional investigation is needed to assess the potential effects of intracellular Ca^{2+} modulation on the pathogenesis and progression of AF.

In patients with persistent AF, an increased prevalence of spontaneous SR Ca^{2+} release events and DADs have also been reported.[235, 240, 241] The activity of single RyR2 channels was found to be increased in patients with cAF.[242, 243] Increased levels of PKA and CaMKII-mediated phosphorylation of RyR2 have been reported in patients and large animal models of cAF.[242, 244, 245] Functionally, however, it appears that mainly CaMKII phosphorylation of RyR2 promotes excessive channel activation and SR Ca^{2+} leak.[243] In addition, reduced interactions of RyR2 with channel-stabilizing subunits such as FKBP12.6 and JPH2 may contribute to increased diastolic SR Ca^{2+} leak and triggered activity.[194, 246] The enhanced SR Ca^{2+} leak is more likely to lead to triggered activity due to upregulation of NCX in patients with cAF.[243]

Finally, AF has been reported in patients with CPVT, which is not surprising since mutant RyR2 channels cause SR Ca^{2+} leak in both the atria and ventricles.[247, 248] Studies in mouse models of CPVT caused by an RyR2 mutant confirmed enhanced SR Ca^{2+} leak in atrial myocytes, consistent with DADs and triggered activity.[249, 250] In addition, atrial conduction slowing has been reported in an RyR2 knock-in mouse model of CVPT, which may be caused by acute Ca^{2+}-dependent inhibition of Na^{+}-channels and a chronic downregulation of Nav1.5 expression.[251, 252] This study suggests a possible mechanistic link between abnormal SR Ca^{2+} release and reduced conduction velocity and a slower action potential upstroke, which might contribute to reentry. Overall, abnormal Ca^{2+} signaling and enhanced diastolic SR Ca^{2+} leak along with cellular DAD-mediated triggered activity may support AF induction by producing DADs and could promote AF persistence by increasing heterogeneity (dispersion) of excitability, thereby causing conduction block that increases the susceptibility to AF-maintaining reentry.

In addition to DADs, late phase-3 EADs have been observed in dogs after rapid atrial pacing (which causes Ca^{2+} loading of the cells) (Figure 7B).[239] This is somewhat surprising since EADs typically occur in the setting of APD prolongation, whereas the atrial APD is usually abbreviated in most models of AF. Several changes favor the development of EADs in cAF, including SR Ca^{2+} leak via RyR2 can promote $I_{Ca,L}$ reactivation, the upregulation of $I_{Na,L}$, and enhancement of I_{NCX}.[243, 253–255] Nevertheless, the potential role of late phase-3 EADs

in the development of AF requires further investigation. Other mechanisms may contribute to the formation of triggered activity, including cytosolic Ca^{2+} alternans (see below), which play a critical role in the initiation of AF in humans.[256]

Ca^{2+}-dependent reentry in AF—Reentry requires a suitable vulnerable substrate, as well as a trigger that acts on the substrate to initiate reentry (discussed above). Atrial remodeling is induced by atrial arrhythmias, and has the potential to increase the likelihood of ectopic activity as well as reentry through multiple mechanisms. The persistence of abnormal Ca^{2+} signaling and enhanced diastolic SR Ca^{2+} leak can activate ion channels and trigger Ca^{2+}-dependent signaling pathways, thereby promoting the evolution of atrial remodeling and the progression of AF to more persistent forms.[257] For example, small-conductance Ca^{2+}-dependent K^+-channels (SK channels) govern the risk of human AF likely by decreasing APD and promoting reentry,[258] and the association between SK channels and RyR2 as the potential internal source of SK channel activation, position SK channels as an important Ca^{2+}-dependent link between triggered activity with reentry.[259, 260] Furthermore, reduced $I_{Ca,L}$ in AF causes APD shortening and promotes reentry.[235] Its reduction is complex and involves downregulation of the Cav1.2 subunit expression by the calcineurin-NFAT system and Cav1.2 breakdown by Ca^{2+}-dependent calpain proteases.[235] Reduced Ca1.3 might also contribute.[261] Reentry-promoting increased I_{K1} may result from a Ca^{2+}-dependent enhancement in expression of Kir2.1-subunits due to a calcineurin-NFAT-mediated decrease in micro-RNA-26.[235]

In atria, the primary mechanism leading to alternans results from abnormalities in Ca^{2+} signaling (Ca^{2+}-driven alternans), with APD alternans being secondary to Ca^{2+}-alternans.[262] Despite some controversies about whether Ca^{2+}-alternans results from fluctuations in SR Ca^{2+} content or from changes in RyR2 refractoriness, Ca^{2+}-alternans can be observed in both animal models of AF and in humans with AF. For instance, SR Ca^{2+} leak increases the susceptibility to Ca^{2+}-alternans and atrial arrhythmias in mice with CPVT mutations in RyR2,[263] and in rabbits with chronic myocardial infarction or hypertension induced atrial remodeling Ca^{2+}-alternans is a prominent feature of the arrhythmogenic substrate.[264, 265] Atrial cardiomyocytes from patients with cAF are also more prone to Ca^{2+}-alternans, an effect which appears to involve an increased activation of adenosine A_{2A} receptors with subsequent enhancement of RyR2-mediated SR Ca^{2+} leak.[266] Overall, computer modeling clearly suggests that elevated Ca^{2+}-driven APD alternans leads to increased arrhythmia vulnerability, complexity, and persistence due to increased heterogeneity of repolarization in atria.[267]

There is evidence that abnormal intracellular Ca^{2+} handling promotes atrial remodeling. Mice with cardiac-restricted overexpression of a repressor form of the cAMP-response element modulator (CREM-Tg mice) develop atrial dilatation, abnormal cardiomyocyte growth, atrial fibrosis along with conduction disturbance leading to spontaneous AF.[268] By crossing the CREM-Tg mice with RyR2-S2814A mice, in which RyR2 phosphorylation by CaMKII is inhibited, the development of a substrate for spontaneous AF was prevented.[269] These studies suggest that RyR2-mediated SR Ca^{2+} leak is involved in atrial remodeling, potentially by activation of calcineurin-NFAT-mediated changes in gene transcription.[269]

In addition, there is emerging evidence that intracellular Ca^{2+} signaling regulates the proliferation and the transition of fibroblasts to collagen-secreting myofibroblasts, thereby promoting reentry.[235] Transient-receptor potential canonical-3 channels (TRPC3) are key mediators of the fibroblast-to myofibroblast transition and their increase in AF involves the NFAT-microRNA-26 pathway.[270] Overall these findings indicate that abnormal RyR2-mediated SR Ca^{2+} leak and the related Ca^{2+}-dependent signaling may drive AF progression via these and possibly other unrecognized remodeling pathways, leading to the evolution of AF-maintaining substrate for reentry (Figure 7).

In summary, despite some controversies about the precise role of RyR2 in AF there is good evidence for contribution of abnormal Ca^{2+} signaling to the formation of the trigger and the substrate for reentry in both animal models and humans with AF.[221] However, it is unknown whether intracellular Ca^{2+} oscillations are required and sufficient to sustain high-frequency foci once the arrhythmia persists. During high-frequency pacing of normal Langendorff-perfused whole rabbit hearts, RyR2 refractoriness initiates SR Ca^{2+}-release alternans in the ventricle without concomitant changes in diastolic SR Ca^{2+} alternans, which points to a potential role of RyR2 dysfunction in Ca^{2+} alternans during pacing.[271] However, in this model RyR2-related Ca^{2+} alternans did not play a major role for the transition of spatially concordant to spatially discordant alternans and the transition of alternans to VF, which rather depended on APD and CV restitution.[271] These findings can be interpreted to suggest that while RyR2-related Ca^{2+} alternans is involved in the initiation of arrhythmias, the maintenance of VF might be less dependent on intracellular SR Ca^{2+}-release oscillations. Of note, studies employing optical mapping of voltage and Ca^{2+} were not yet performed in the diseased ventricle or atrium, thus the consequences of dysfunctional RyR2 (and other ECC components) for arrhythmia maintenance, particularly in the diseased atrium, remain unknown and require thorough addressing in subsequent studies. Simultaneous high resolution optical mapping of voltage and Ca^{2+} in perfused intact human atria of sinus rhythm and AF patients should be performed to obtain first hints about the putative role of intracellular Ca^{2+} oscillations for the fibrillatory process during pacing-induced AF.[272]

THERAPEUTIC APPROACHES TO CORRECTING CALCIUM MISHANDLING

Ca^{2+}-handling within cardiomyocytes has been recognized as a potential target for the treatment of cardiac disease for a long time. One class of drugs known as 'Ca^{2+} channel antagonists' target the voltage-gated sarcolemmal Ca^{2+} channels, and are currently being used clinically to treat hypertension, angina pectoris, cardiomyopathy, and cardiac arrhythmias. Fleckenstein described the first Ca^{2+} channel blockers as new drugs for the treatment of coronary disease about 50 years ago.[273] During decades of subsequent studies, the role of Ca^{2+} channels in cardiac muscle contraction was elucidated [for review, see[274]]. Moreover, the biophysical and genetic identities of various voltage-gated Ca^{2+} channels were subsequently described.[275, 276] Several classes of antagonists have been described (i.e., benzothiazepines, phenylalkylamines, and dihydropyridines), and are now part of the formulary for the treatment of cardiac diseases including arrhythmias. Ca^{2+} channel blockers are able to decrease the automaticity of ectopic foci in the heart and have emerging uses in a number of arrhythmia. For example, T-type Ca^{2+} channel blockers and LTCC blockers have efficacy in reducing AF arrhythmia burden and can prevent electrical remodeling.[277–279]

Moreover, while mainstay for treatment of CPVT is beta-blockade, there has been early evidence that also blocking $I_{Ca,L}$ with the LTCC blocker verapamil prevented ventricular arrhythmias.[279]. Overall, it is believed that reduced $I_{Ca,L}$ results in less Ca^{2+} overload of the myocyte, reducing predisposition to ectopy which can trigger arrhythmias.

During the past 15 years or so, several groups including our own have tried to develop pharmaceutical agents that target the intracellular Ca^{2+} release channel. To our knowledge, the first example of an experimental small molecule is K201 (also referred to as JTV519), a 1,4-benzothiazepine shown to normalize RyR2 gating in a canine model with tachycardia-induced HF.[280] Subsequently, K201 was shown to prevent lethal ventricular tachyarrhythmias in a mouse model of CPVT by stabilizing RyR2 channels.[281] Studies using recombinantly expressed RyR2 channels with CPVT-linked missense mutations showed that K201 can normalize mutant channel activity.[282] In addition, K201 was shown to exert anti-arrhythmic effects against AF in an experimental guinea pig model.[283] Although K201 normalizes defective RyR2 channels, this compound also inhibits various other targets including annexin V and K^+ channels, raising concerns about potential off-target and pro-arrhythmic side-effects.[284, 285] The proposed mechanism of RyR2 stabilization, through normalization of the binding stoichiometry of RyR2 and FKBP12.6, remains controversial. For example, the role of FKBP12.6 in reducing RyR2-mediated Ca^{2+} leak has been debated and this topic has been robustly reviewed.[77, 286] Further, dissociating FKBP12.6 from RyR2 by FK506 did not affect suppression of spontaneous Ca^{2+} release events in rat ventricular myocytes questioning the role of FKBP12.6 binding in the mechanism of FK506.[287] While debate exists in the field, there is a preponderance of datam, which suggests that RyR2 stabilization can be achieved by small molecules. Since discovery of this first molecule, newer generations of RyR2 stabilizing molecules have been developed. For example, a 1,4-benzothiazepine named S107 - a more specific RyR2-blocker - was shown to prevent ventricular arrhythmias in a CPVT mouse model heterozygous for mutation R2474S.[80] Moreover, S107 has been shown to inhibit the RyR2-mediated diastolic SR Ca^{2+} leak in atrial myocytes in a number of *RYR2* mutation knock-in models and decreased the incidence of burst pacing induced AF.[250] While believed to have less off-target effects on a host of other receptors than K201, there have yet to be comprehensive studies on the major ion channels responsible for EC-coupling and Ca^{2+} homeostasis.[80]

Although flecainide is a class IC anti-arrhythmic drug with Na^+ channel blocking properties, this drug has also been shown to inhibit RyR2 and exert therapeutic effects in mouse models of and patients with CPVT.[288] This is most salient in patients for whom beta blockade is less effective. For example, flecainide has been shown to inhibit aberrant RyR2 activity and reduce spontaneous Ca^{2+} waves in both patients with CPVT refractory to beta blocker therapy and in Casq2−/− mouse models of CPVT in numerous independent studies.[289–291]. While the suppression of aberrant SR Ca^{2+} release seems a consistent effect of flecainide, other investigators have questioned whether RyR2 is the primary molecular target. For example, increasing the threshold for triggered activity by action on the cardiac Na^+ channels with minimal effect on intracellular Ca^{2+} flux has been proposed.[291] In addition, reducing elevated intracellular Ca^{2+} levels through reduction of I_{Na} leading to increased net Ca^{2+} influx via NCX has also been proposed.[292] In an attempt to reconcile these various mechanisms, a so-called “triple mode of action” of flecainide has been proposed whereby all

these various mechanisms are incorporated with the predominant effect being reduction of spontaneous Ca^{2+} release from RyR2.[293, 294]

In addition to these molecules, other classes of RyR2 inhibitors with anti-arrhythmic effects have been described, including dantrolene,[295] carvedilol analogues,[296] and tetracaine derivatives.[297] In addition to LTCC and RyR2, other Ca^{2+} channels, Ca^{2+} transporters, and Ca^{2+}-dependent signaling molecules (such as CaM, CaMKII) are potential therapeutic targets.

CONCLUDING REMARKS

The last 3 decades have seen a remarkable expansion in identifying the genetic and molecular etiologies of both congenital and acquired cardiac arrhythmias. While the specific molecular mechanisms of arrhythmic remodeling of the heart are as diverse as the many ways in which arrhythmia can present, Ca^{2+} is a critical and central player in many. Progress into identifying the role of Ca^{2+} in arrhythmias has led to novel understanding of the physiologic and pathologic regulation of the cardiomyocyte. When coupled to rapid advancement in genetic sequencing platforms, and recent breakthroughs in the development of both *in vitro* and *in vivo* models of disease, these advances offer the possibility of revolutionizing the diagnosis and treatment of these common and potentially life-threatening conditions.

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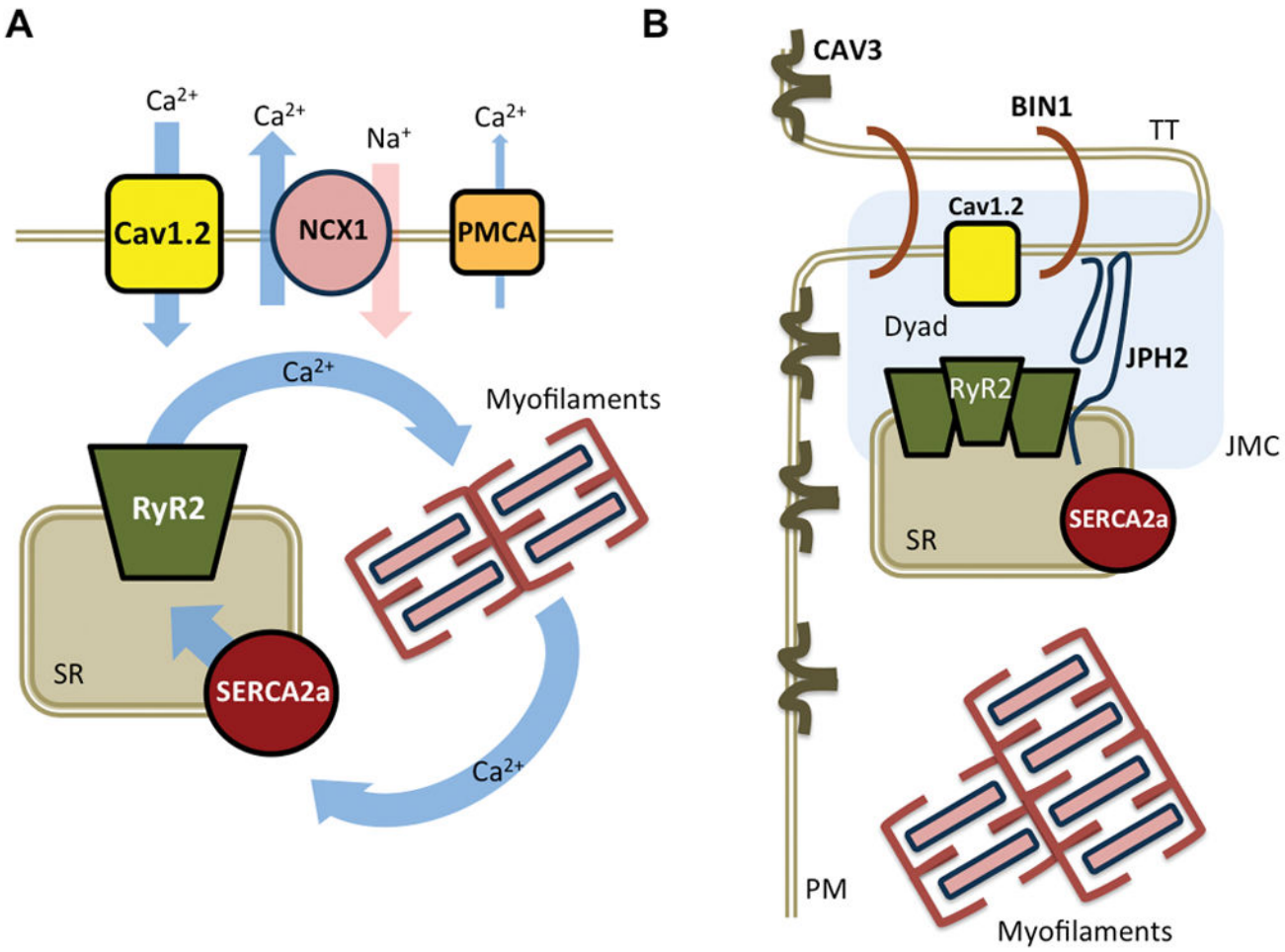

Figure 1. Role of calcium-handling in excitation-contraction (EC) coupling

A. Schematic overview of key Ca^{2+}-handling proteins involved in EC coupling. **B.** Schematic diagram of Ca^{2+} the release unit and major components of the JCM. The transverse tubule (TT) and SR membranes approximate to form the dyad. BIN1, bridging integrator 1; Cav1.2, L-type Ca^{2+} channel; CAV3, caveolin-3; JMC, junctional membrane complex; JPH2, juncophilin-2; NCX1, Na^{+}/Ca^{2+} exchanger; PM, plasma membrane; PMCA, plasmalemmal Ca^{2+}-ATPase; RyR2, ryanodine receptor type-2; SERCA2a, sarco/endoplasmic reticulum ATPase type-2a; SR, sarcoplasmic reticulum.

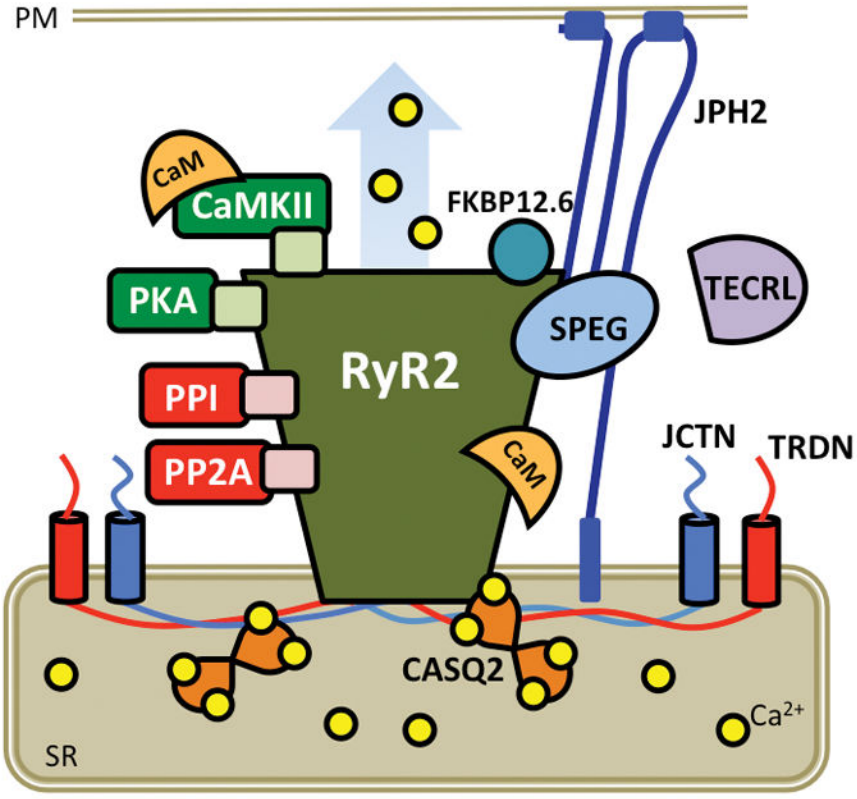

Figure 2. RyR2 macromolecular complex

Cartoon representing RyR2 pore-forming subunits with accessory proteins that bind to and/or modulate channel function. CaM, calmodulin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CASQ2, calsequestrin-2; FKBP12.6, FK506-binding protein 12.6; JCTN, junctin; JPH2, juncophilin-2; PKA, protein kinase A; PM, plasma membrane; PP, protein phosphatase; SR, sarcoplasmic reticulum; TECRL, trans-2,3-enoyl-CoA reductase-like protein; TRDN; triadin.

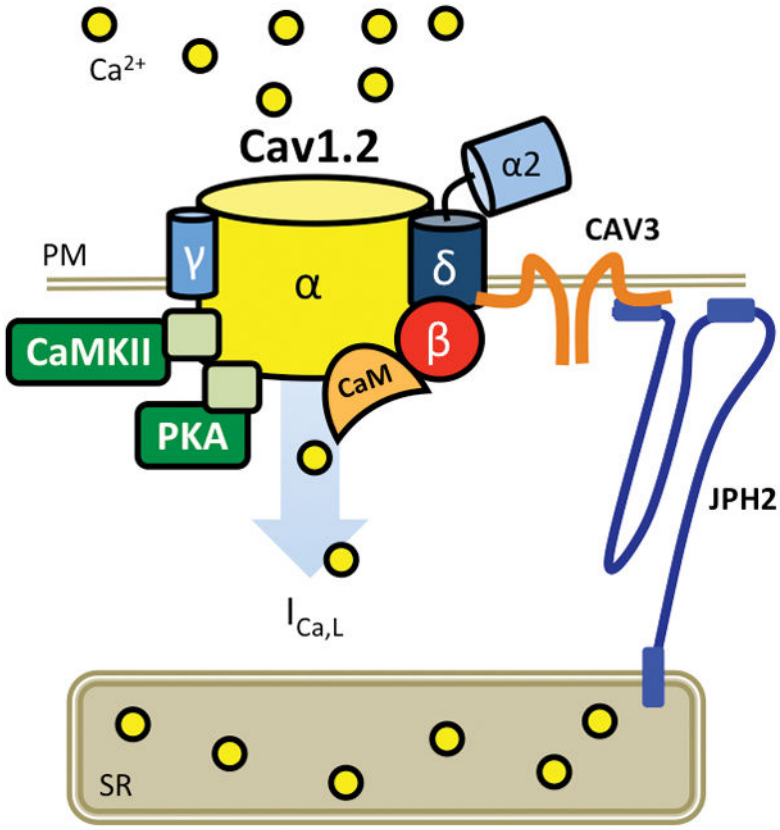

Figure 3. LTCC macromolecular complex

Cartoon representing Cav1.2 pore-forming β subunit with accessory β2, β, γ, δ subunits. CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CAV3, caveolin-3; $I_{Ca,L}$, L-type Ca^{2+} current; JPH2, juncophilin-2; PKA, protein kinase A; PM, plasma membrane; PP, protein phosphatase; SR, sarcoplasmic reticulum.

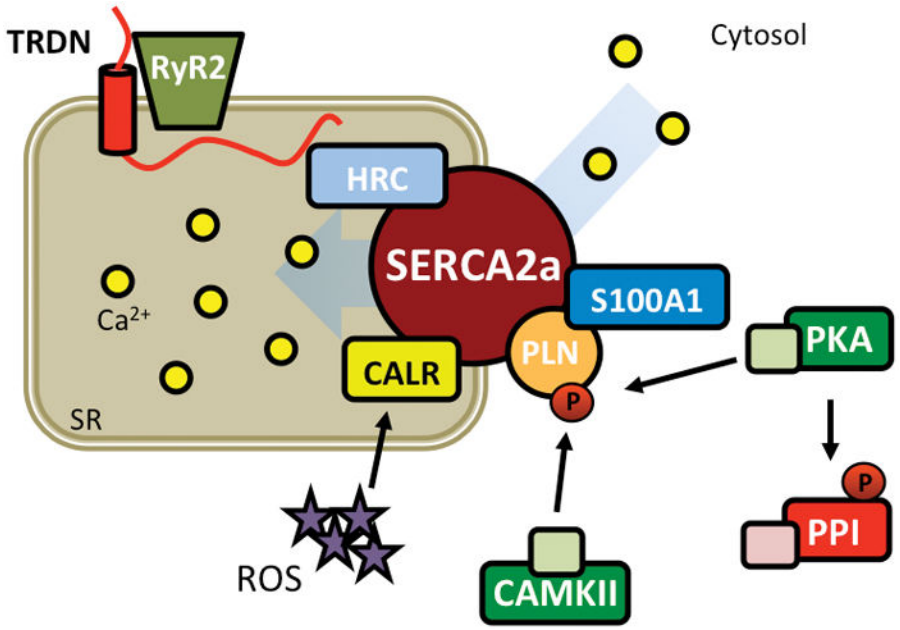

Figure 4. SERCA2a macromolecular complex

Cartoon representing SERCA2a complex required for reuptake of $Ca2^{+}$ from the cytosol to the SR. CALR, calreticulin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; HRC, histidine-rich Ca^{2+} binding protein; PKA, protein kinase A; PP, protein phosphatase; ROS, reactive oxygen species; RyR2, ryanodine receptor type-2; SERCA2a, sarco/endoplasmic reticulum ATPase type-2a; SR, sarcoplasmic reticulum; TRDN, triadin

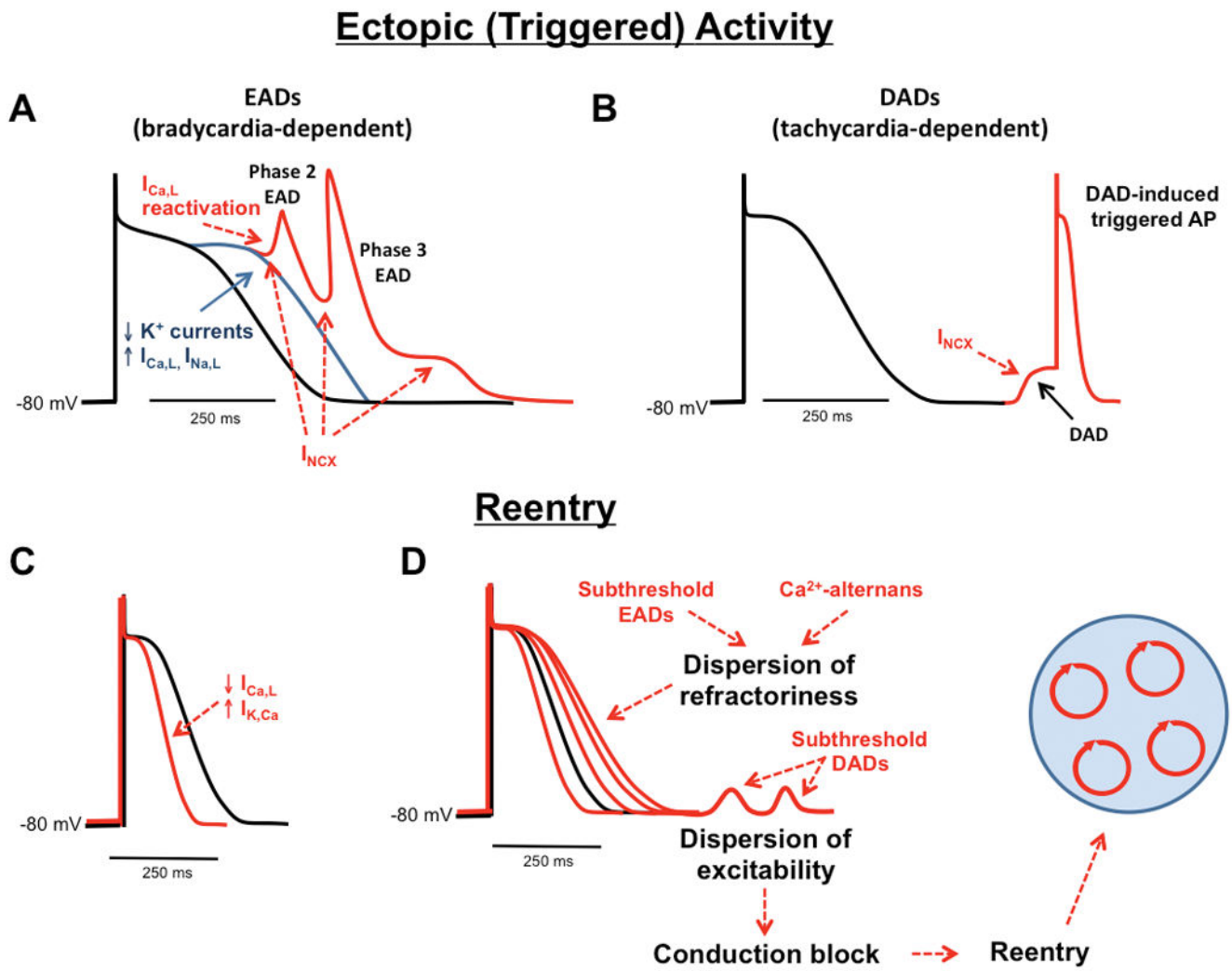

Figure 5. Key electrophysiological mechanisms leading to cardiac arrhythmias

Ectopic (triggered) activity is primarily caused by **A**) early after-depolarizations (EADs) that occur mainly during bradycardia or following a pause, and **B**) delayed after-depolarizations (DADs) that occur using during tachycardia. Reentry requires a vulnerable substrate, which can be caused by **C**) action potential shortening or **D**) dispersion of refractoriness. $I_{Ca,L}$, L-type Ca^{2+} current; $I_{K,Ca}$, Ca^{2+} dependent K^+ current; $I_{Na,L}$, late Na^+ current; I_{NCX}, Na^+/Ca^{2+} exchanger current;

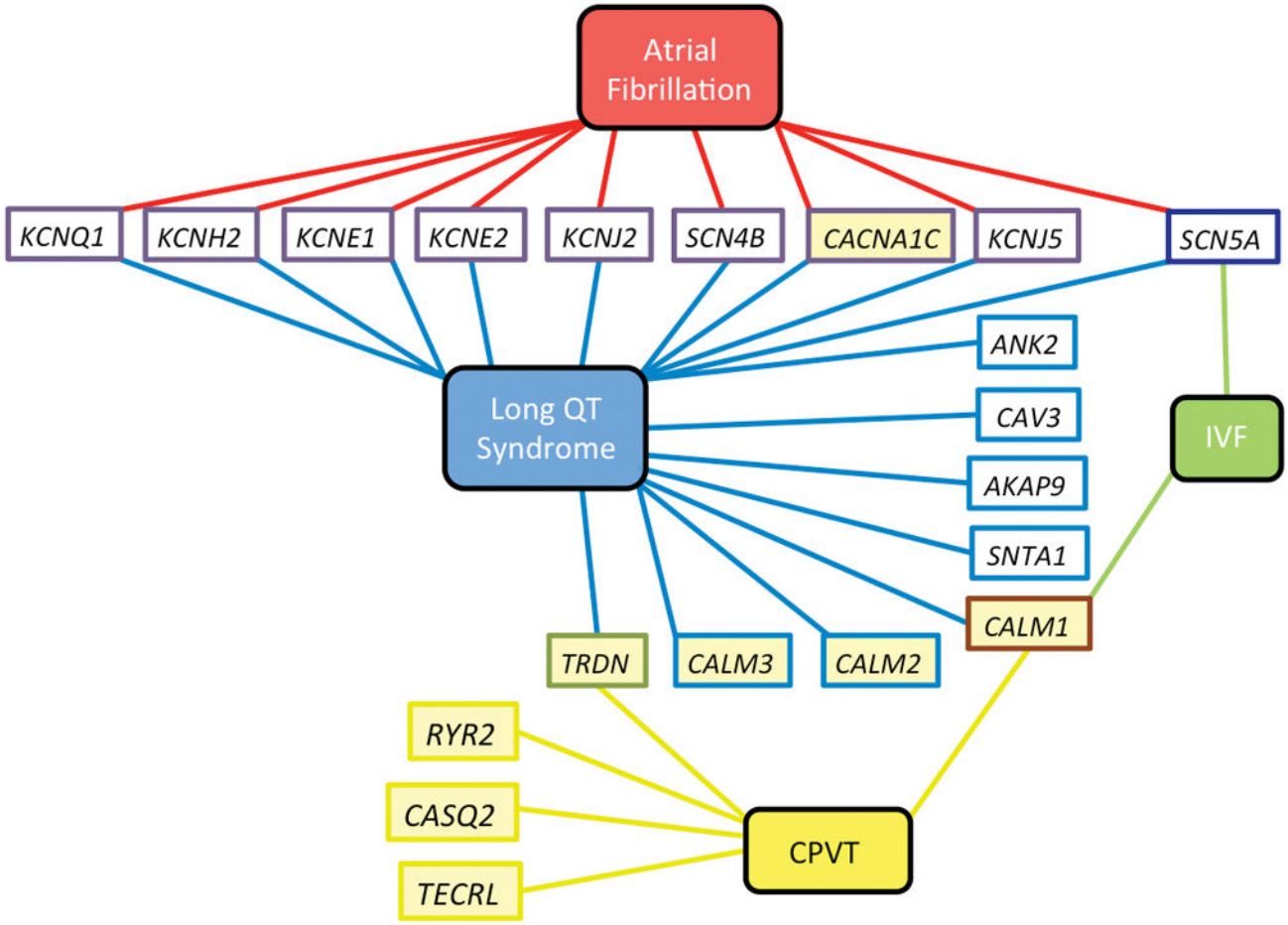

Figure 6. Diagram showing which genes have been linked to genetic arrhythmia disorders

Yellow fill indicates gene that encodes a Ca^{2+}-sensitive or Ca^{2+}-handling protein. CPVT, catecholaminergic polymorphic ventricular tachycardia; IVF, idiopathic ventricular fibrillation.

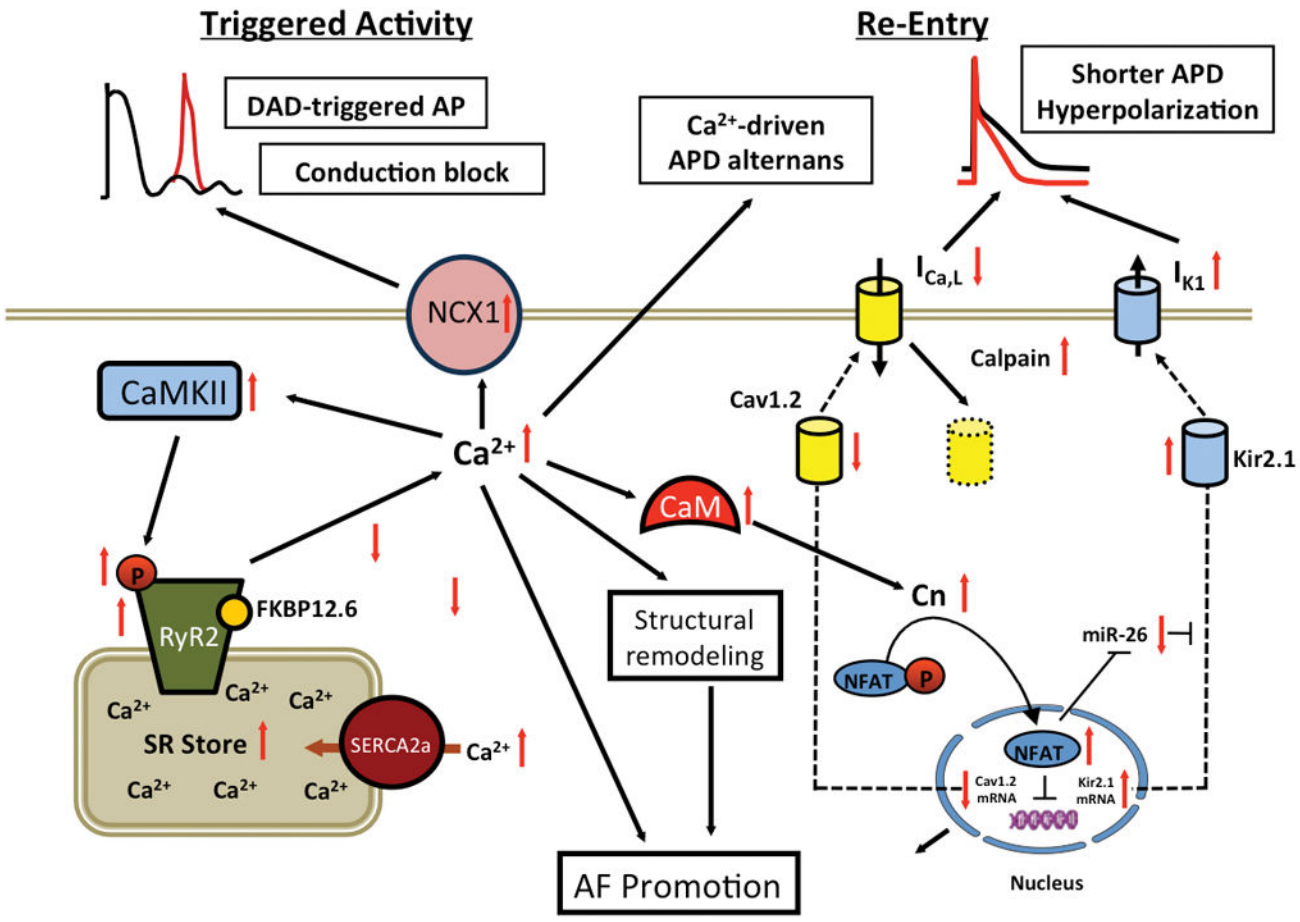

Figure 7. Calcium-dependent arrhythmia mechanisms in atrial fibrillation (AF)

Schematic diagram delineating which changes in intracellular Ca^{2+}-handling promote arrhythmia mechanisms leading to AF. Enhanced RyR2-mediated Ca^{2+} release leads to activation of NCX, which in turn can cause a DAD-mediated triggered action potential (AP). Shortening of the AP duration (APD) due to reduction of $I_{Ca,L}$ (L-type Ca^{2+} current) and membrane hyperpolarization due to upregulation of $I_{K,1}$ (inward rectifier K^+ current) promote reentry. CaM, calmodulin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Cav1.2, L-type Ca^{2+} channels; Cn, calcineurin; FKBP12.6, FK506-binding protein 12.6; miR-26, micro-RNA-26; mRNA, messenger RNA; NFAT, nuclear factor of activated T-cells; RyR2, ryanodine receptor type-2; SERCA2a, sarco/endoplasmic reticulum ATPase type-2a.

Table 1

Summary of CPVT-associated genes

Type	MIM*	Gene	Protein	Genetic Locus	Frequency	Inheritance
CPVT 1	604772	*RYR2*	Ryanodine receptor 2	1q42.1-q43	50–60%	AD
CPVT 2	611938	*CASQ2*	Calsequestrin 2	1p13.1	Rare	AR
CPVT 3	614021	*TECRL*	Trans-2,3-enoyl-CoA reductase-like	7p22-p14	Rare	AR
CPVT 4	614916	*CALM1*	Calmodulin 1	14q31-q32	Rare	AD
CPVT 5	603283	*TRDN*	Triadin	6q22.31	Rare	AR

*Phenotype MIM number; AD, autosomal dominant; AR, autosomal recessive

Table 2

Summary of LQTS-associated genes

Type	MIM*	Gene	Protein	Genetic Locus	Frequency	Inheritance
LQTS 1	192500	*KCNQ1*	Kv7.1	11p15.5-p15.4	30–35	AD
LQTS 2	613688	*KCNH2*	KV11.1	7p36.1	25–30	AD
LQTS 3	603830	*SCN5A*	NaV1.5	3p22.2	5–10	AD
LQTS 4	600919	*ANK2*	Ankyrin B	4q25-q26	Rare	AD
LQTS 5	613695	*KCNE1*	MinK	21q22.12	Rare	AD
LQTS 6	613693	*KCNE2*	MinK related protein 1	21q22.12	Rare	AD
LQTS 7	170390	*KCNJ2*	Kir2.1	17q24.3	Rare	AD
LQTS 8	**601005**	***CACNA1C***	**CaV1.2**	**12p13.33**	**Rare**	**AD**
LQTS 9	611818	*CAV3*	Caveolin 3	3p25.3	Rare	AD
LQTS 10	611819	*SCN4B*	Sodium channel β4	11p23	Rare	AD
LQTS 11	611820	*AKAP9*	Yotiao	7p21.2	Rare	AD
LQTS 12	612955	*SNTA1*	Syntrophin α1	20q11.21	Rare	AD
LQTS 13	613485	*KCNJ5*	Kir3.4	11q24.3	Rare	AD
LQTS 14	**616247**	***CALM1***	**Calmodulin 1**	**14q32.11**	**Rare**	**AD**
LQTS 15	**616249**	***CALM2***	**Calmodulin 2**	**2p21**	**Rare**	**AD**
LQTS 16	**114183**^	***CALM3***	**Calmodulin 3**	**19q13.32**	**Rare**	**AD**
LQTS 17	**603283**^	***TRDN***	**Triadin**	**6q22.31**	**Rare**	**AR**

*Phenotype MIM number;

^Gene MIM number; AD, autosomal dominant; AR, autosomal recessive; lines in bold are Ca^{2+}-sensitive proteins or involved in Ca^{2+}-signaling